ABSTRACT

Title of Thesis:	THE EFFECTS OF COAL ASH STORAGE FACILITIES ON AQUATIC BIOTA
	Shannon Michelle Edmonds, Master of Science, 2018
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Dry storage of coal combustion wastes (CCW) can negatively impact the surrounding environment. CCW leachates are characterized by low pH and high conductivity and are often enriched with metals and metalloids that threaten nearby ecosystems. In the present study, a quarterly series of whole effluent toxicity (WET) tests were completed on facility-treated discharge samples from three Maryland CCW disposal facilities. Priority metals and major ions were measured over time and concurrently with bioassays. Chronic toxicity was identified at all three locations at varying frequencies. The wastewaters were consistently high in total dissolved solids (TDS) with high conductivities, evidence that major ion toxicity could be contributing to the effects. Traditional Phase I Toxicity Identification Evaluation methods do not address these issues. Therefore, the present study evaluated the toxicity associated with major ion imbalances using mock effluents and a weight-of-evidence approach in order to identify the primary causative ions.

THE EFFECTS OF COAL ASH STORAGE FACILITIES ON AQUATIC BIOTA

by

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Executive Summary

This project was initiated for the purpose of investigating the toxicity associated with the discharge effluents from three Maryland coal ash facilitates: the Brandywine, Faulkner, and Westland Ash Management Facilities. These are considered dry storage facilities where coal ash is held in a landfill (monofill), and each now holds greater than 750,000 cubic meters of Coal Combustion Waste (CCW). The storage facilities are associated with coal-fired power plants and have been in operation since the 1970s. They were unlined when constructed and capped with vegetation when they reached capacity. The 2008 Maryland Department of the Environment (MDE) state legislation, as well as 2015 US Environmental Protection Agency (USEPA) national legislation, required the installation of leachate treatment systems within each facility. These systems are a series of active and passive treatments designed to remove or reduce contaminants to acceptable limits. The leachates are pumped, treated, and then held in leachate storage ponds, where they are released as effluents into nearby streams. The treated effluent at each facility is associated with a National Pollution Discharge Elimination System (NPDES) permit that requires monitoring specific analytes of these effluents upon discharge and quarterly whole effluent toxicity (WET) testing that began in May 2017.

The overall purpose of this thesis is to describe the results of the quarterly toxicity testing between May 2017 and September 2018 and to use the corresponding chemical analyses of the effluents measured at each site to make informed judgments on the nature of the effluent toxicity. A preliminary Toxicity Identification Evaluation

(TIE) was undertaken that involved the creation of a synthetic effluent in order to identify the cause of toxicity.

Daily, weekly, and monthly analysis of metals, major ions, and water quality characteristics of the post-treatment discharge ponds was completed by Microbac Laboratories Inc. in Baltimore, MD. These analyses correspond to the frequency of discharge, i.e., the daily analytes and parameters were measured each time the effluent was discharged. The weekly analytes and parameters were measured once weekly during discharge, and the monthly analytes and parameters were measured once per calendar month during discharge. The list of analytes required at each facility by MDE was limited in nature and therefore insufficient to make comprehensive decisions as to what was potentially causing toxicity. The timing of the toxicity testing also affected the extensiveness of analyses; quarterly toxicity tests that were contemporaneous with monthly discharges therefore have the most data. Expected analytical results were understood to be the responsibility of the facilities and the project was designed with the intention of having certain analyses that did not occur. We are still in the process of trying to get the analytical results for the most recent effluent discharges.

The effluents of the three facilities varied in their toxicity to *Ceriodaphnia dubia* and fathead minnow (*Pimephales promelas)*. The Brandywine treated effluent had measurable toxicity every quarter it was tested. The Faulkner treated effluent had measurable toxicity on two out of six quarters. The Westland treated effluent had measurable toxicity three out of six quarters. A subsequent series of tests was undertaken to serve as a pilot TIE of the Brandywine effluent. This was a preliminary

investigation meant to inform the process going forward and was not intended to be comprehensive or to fulfill the requirements of the USEPA three phase TIE.

The available chemical analysis data, and the high conductivity and total dissolved solids (TDS) pointed to the possibility of a major ion imbalance as the cause of toxicity. In addition, *C. dubia* were generally found to be more sensitive to the effluent than the fathead minnow, consistent with expected sensitivity patterns for the two species. Major ions are part of a class of toxicants that remains difficult to address and determine and are not characterized through traditional TIE Phase I USEPA manipulations. Previous methods applied utilize tiered approaches and the creation of synthetic effluents prepared to mimic the ionic composition of the effluent. The principal methodology of this approach was to compare toxicity between the synthetic effluent and the actual effluent and use the results to identify or rule-out particular ions as a cause of toxicity. The mock effluent created to mimic the treated effluent at Brandywine in ionic composition was similar in toxicity to the Brandywine effluent, supporting the hypothesis that major ion toxicity could be the overall cause of toxicity. Subsequent tests designed to target chloride, potassium, and sulfate were unsuccessful at identifying or ruling-out any individual ion. It was demonstrated that potassium toxicity is ameliorated by the presence of other ions, especially sodium, and that the mechanisms of toxicity for ionic mixtures are complex. Suggestions for future research include the continued quarterly monitoring of toxicity at each facility to determine the potential impact of seasonality and to establish trends within each facility in order to better predict and assess toxicity.

This first chapter of this thesis serves as introductory and background information. The second chapter explains the results of quarterly WET testing at each facility in order to determine toxicity. The third chapter is the preliminary investigation into the cause of toxicity by the creation of synthetic effluents and a TIE approach. The fourth chapter serves as an overall summery, an elaboration on the recommendations for future TIE efforts, and site recommendations for each facility.

Dedication

This thesis is dedicated to my parents, Marylynn and Michael, for their unwavering love and support. To my sister, Lauren, for her encouragement and insightfulness. To Joseph Paskewicz for his devotion, perpetual patience, and emotional support. Lastly, in memory of my twin brother, Brian, whose example and keen interest in science encouraged and inspired me throughout his life and continues to motivate me today.

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I would like to express my great appreciation to Dr. Lance Yonkos for his valuable and constructive guidance and continual confidence throughout my graduate assistantship. My committee, Dr. Daniel Fisher and Dr. Carys Mitchelmore for their instructions and insight. My lab, Rachel Harrison and Alex MacLeod for their encouragement, advice, and camaraderie. Rachel Eberius, Dr. Meredith Bohannon, and Dr. Tyler Frankel for their inspiration, assistance, and friendship. Lastly, Dr. William Bowerman for the gracious opportunity to learn and experience something new.

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List of Abbreviations

ANOVA- Analysis of Variance

CCB- Coal Combustion Byproducts

CCW- Coal Combustion Waste

CWA- Clean Water Act

DMW- Diluted Mineral Water

DO- Dissolved Oxygen

EDTA- Ethylenediaminetetraacetic acid

FBC- Fluidized Bed Combustion

FGD- Flue Gas Desulphurization

GFF- Glass Fiber Filtration

IC25- Inhibition Concentration 25%

ICP/MS- Inductively Coupled Plasma Mass Spectrometry

MDE- Maryland Department of the Environment

NOEC- No Observable Effect Concentration

NPDES- National Pollution Discharge Elimination System

PAH- Polycyclic Aromatic Hydrocarbon

PMSD- Percent Minimal Statistical Difference

RO- Reverse Osmosis

SPE- Solid Phase Extraction

TDS- Total Dissolved Solids

TIE- Toxicity Identifcation Evaluation

TRE- Toxicity Reduction Evaluation

USEPA- United States Environmental Protection Agency

WET- Whole Effluent Toxicity

WMU- Waste Management Unit

WQC- Water Quality Criteria

YCT- Yeast Cerophyll Trout Chow

Chapter 1 : Introduction

Introduction

In 2015 the United States produced 117.3 million tons of coal combustion waste (CCW), or coal ash (American Coal Ash Association, 2017). Approximately 60% of this waste was "beneficially used" and the remainder was stored in waste facilities. Beneficial use includes the use in concrete, cement, structural fill, soil stabilization, and agriculture (Chindaprasirt et al., 2009; Jayaranjan et al., 2014). A 2010 report estimated that 300 landfills and 584 storage ponds exist throughout the United States (Zierold & Sears, 2015) These facilities vary in size and construction, and can be unlined, clay-lined, or composite lined (USEPA, 2010). Coal ash related contaminants can runoff and seep from unlined landfills. Leachates generated are high in trace elements and can elevate contaminant levels in groundwater, surface water, and soil (Baba et al., 2008). Many of the major coal ash constituents are carcinogenic, neurotoxic, and mutagenic (Chakraborty & Mukherjee, 2009). Therefore, contamination of ground and surface water surrounding coal ash storage facilities threatens the local ecosystem as well as public health. Understanding the risks associated with coal combustion waste sites is critical for identifying appropriate remediation techniques.

The state of Maryland is home to 31 coal ash monofills and minefills. A monofill is a landfill that contains only one type of waste, such as coal ash, and is therefore the technical term used to refer to coal ash landfills (Figure 1.1). In 2008 a $54 million lawsuit was settled between Constellation Energy and residents of Gambrills, MD following the discovery of As, Pb, Cd, and SO_4 in wells near a CCW

disposal site (Luther, 2011). Statewide concern of coal ash storage facilities and their effect on the environment and surrounding ecosystems increased and prompted the development of statewide regulations by the Maryland Department of the Environment (MDE) that were made effective on December 1, 2008 (MDE, 2009). The new regulations identified rules for monitoring, zoning, and waste management, and required compliance by solid waste landfills. Additionally, provisional regulations were made requiring dust control, post-closure monitoring, and annual reporting (MDE, 2009). In 2013 efforts were initiated to clean up three Maryland facilities: the Brandywine, Faulkner, and Westland coal ash storage sites (Wheeler, 2013). These facilities have historically been associated with local stream contamination and have been identified by the United States Environmental Protection Agency (U.S. EPA) as "potential damage cases" (Environmental Integrity Project, 2013). All three facilities have exceeded chronic water quality limits for various metals (Cd, Fe, Al, Mn, SO_4) in ground and surface water surrounding the sites. The three sites are currently managed by NRG Energy Incorporated and have faced enforcement actions by MDE for violations of the Clean Water Act which amounted to $110,000 in penalties for the period of October 2016 through September 2017 (MDE, 2018).

Figure 1.1 Westland Coal Ash Monofill

The Brandywine Ash Storage Site covers 140 acres bordering the Patuxent River at Swanson's Creek in Prince George's County, Maryland and is associated with the Chalk Point Generating Station. The facility was built and began receiving ash in 1971. The facility maintains four cells; one in operation and used for coal storage, and three with historic coal ash that are not currently operational and are capped with soil and vegetation (Figure 1.2). A geosynthetic closure capping system was installed in 2018 to comply with new regulations.

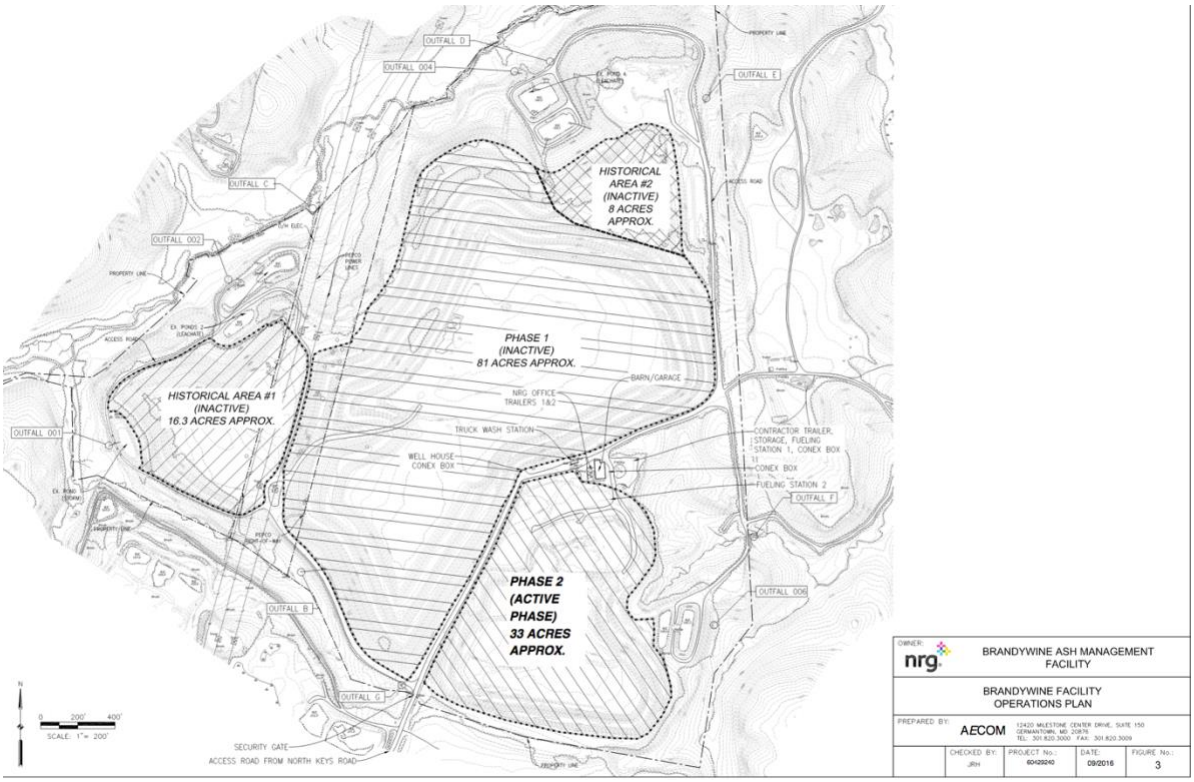

Figure 1.2 Brandywine Coal Ash Storage Site Map (AECOM, 2016b)

The Faulkner Ash Storage Site spans 180 acres in Charles County and is located on the Potomac River (Figure 1.3). Faulkner is in close proximity to the Zekiah Swamp, an important habitat for a number of species (Burton & Pinkney, 1994; Ciccotto & Stranko, 2009). According to Faulkner's 2016 Coal Combustion Byproduct (CCB) Tonnage Report, flyash at the Morgantown Generating Station, the plant next to Faulkner, is processed onsite and then sold for beneficial use in concrete production. The bottom ash generated at Morgantown in is transported to Brandywine for storage. Faulkner is currently not storing newly processed ash, but instead managing historic wastes.

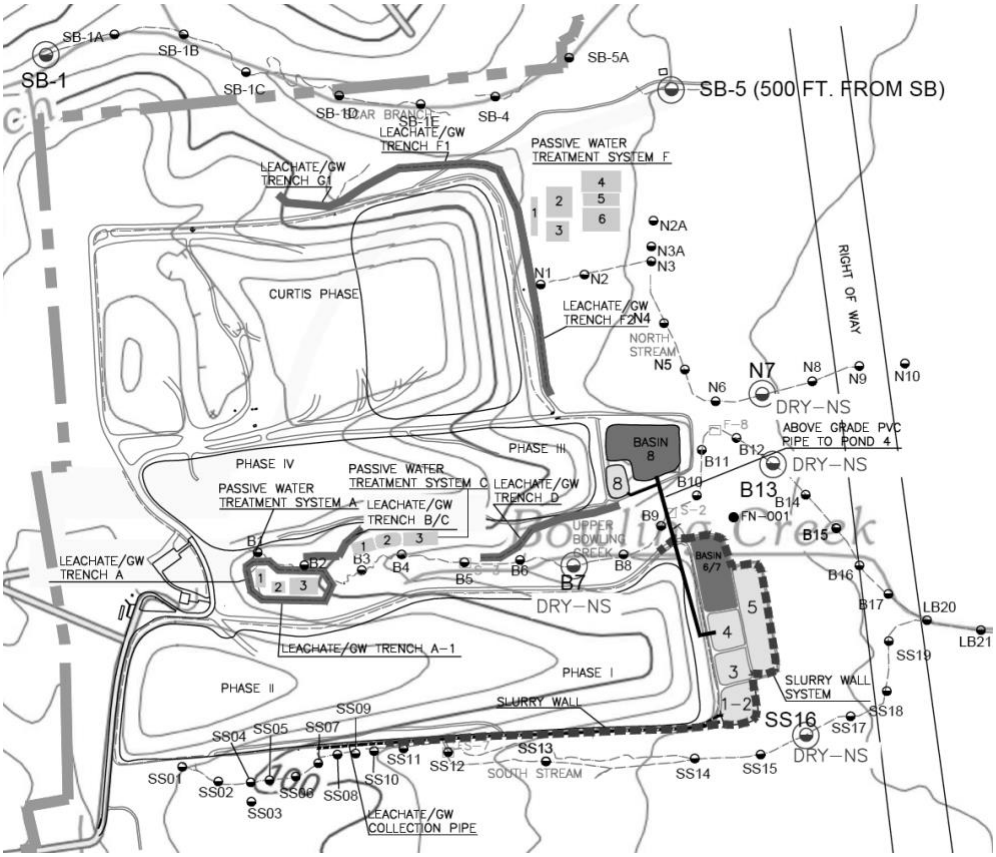

Figure 1.3 Faulkner Coal Ash Storage Facility (provided by Ann Wearmouth)

The Westland Ash Storage Site (henceforth referred to as Westland) is an 83-acre facility that receives ash from the Dickerson plant located in Montgomery County, MD. The facility was constructed in 1979 and is composed of three cells, with only one in current operation (Figure 1.4). According to Dickerson's 2016 CCB Tonnage Report, all of the flyash and bottom ash generated in 2016 was disposed of onsite.

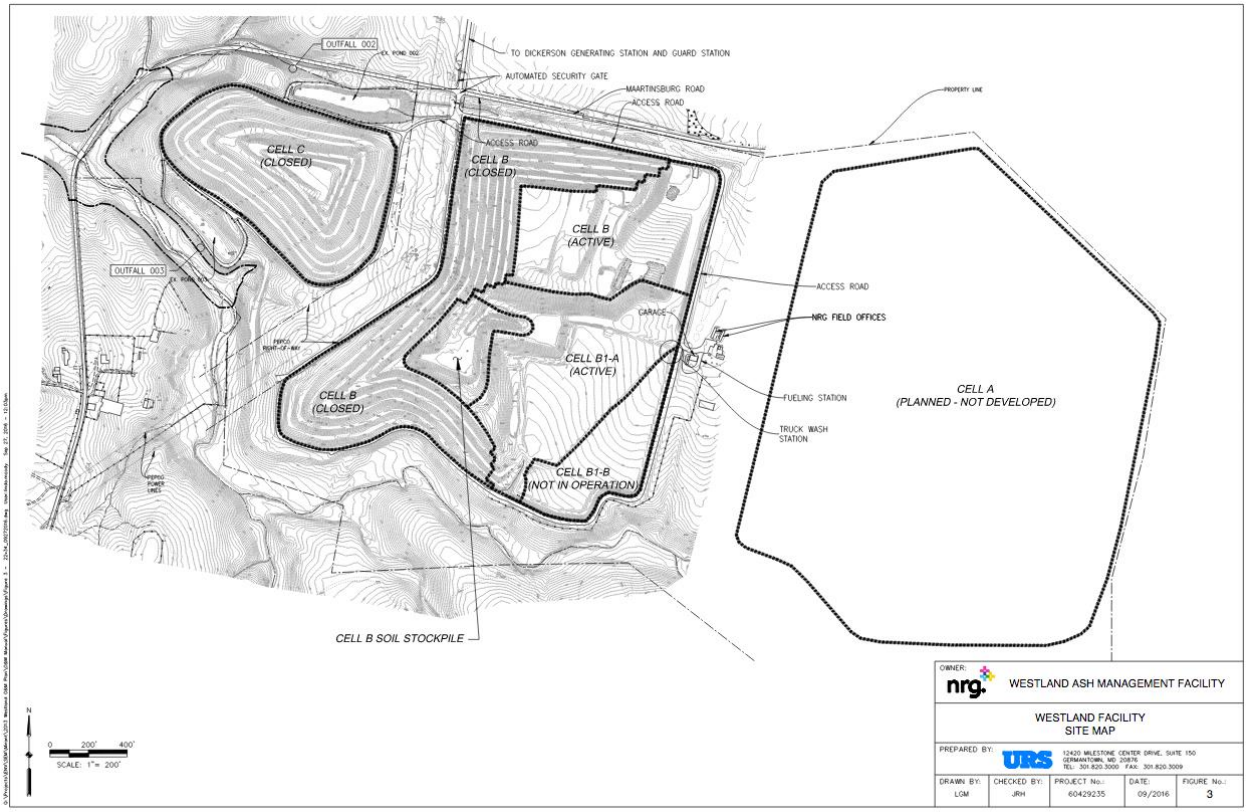

Figure 1.4 Map of Westland Coal Ash Storage Facility (AECOM, 2016a)

Coal Ash Storage

Coal combustion solid waste includes fly ash, bottom ash, boiler slag, flue gas desulphurization (FGD) residues, and fluidized bed combustion (FBC) wastes, which are collectively referred to as coal combustion waste (CCW). All coal-fired plants generate some of these wastes (USEPA, 2010). The waste generated may be primarily dry or primarily wet, depending on the combustion technology utilized. Dry material is disposed in landfills, and wet material is deposited in surface impoundments. Both landfills and impoundments are collectively referred to as waste management units (WMUs) (USEPA, 2010). Waste management units vary in the types of liner employed; clay-lined units utilize clay to impede leachate flow, while composite-lined units are constructed from layered man-made materials designed to significantly

inhibit leachate flow. Unlined WMUs place CCWs directly in surrounding soils (USEPA, 2010).

Properties of CCW are highly variable and dependent on the type of coal used and the temperature at which it was combusted. Sub-bituminous and lignite coals usually contain 5-15% calcium oxide and are more cementitious than bituminous and anthracite coal, which contain less than 5% calcium oxide and are not cementitious (Asokan et al., 2004). The characteristics of coal ash held in wet storage facilities is also affected by the ash slurry flow rate, the size and shape of the storage pond, and the particle size of the ash (Asokan et al., 2004). Metal speciation in coal ash can also vary depending on the region that the coal originated (Sajwan et al., 2006). For example, chromium(VI), the toxic valence state is enriched in 10-30% of western U.S. coal fly ash, compared with chromium(III) which is characteristic of eastern U.S. coal fly ash (Sajwan et al. 2006). Environmental setting, location, and climate can affect the likelihood and rate of leachates entering the environment (USEPA, 2010). Modeling and understanding the risks associated with coal ash storage facilities is highly site-specific because of the variations in the type of coal stored and in the facility design.

Fly ash is the lightest coal combustion waste and is collected by filtering the flume gas during combustion (Sajwan et al., 2006). Particle sizes range from 0.1 to 1 μm, and have of rough surfaces to which contaminants can adhere. Coal fly ash is mainly comprised of Ca, Mg S, Al, Na, and Fe (Palmer et al., 2000). Fly ash is a pozzolanic material, meaning that it is siliceous and can react with CaO when wet, forming a cement-like material (Palmer et al., 2000). Silicon dioxide is a principle

component of fly ash, the percent by weight varies depending on the type of coal combusted (Jayaranjan et al., 2014). Class F fly ash is the product of anthracite and bituminous coals and is <8% CaO. Class C fly ash is produced by lignite and sub-bituminous coals and is >8% CaO. Class C typically contains more than 8% calcium oxide whereas class F typically contains less than 8% calcium oxide (Furlong & Hearne, 1994; Jayaranjan et al., 2014). Both can be used as additives to cement and the general classification system allows for wide differences within each class, making it difficult to define how each class reacts in concrete (Halstead & Crumpton, 1986).

Bottom ash is uncombusted material collected from the bottom of the boiler. Bottom ash particulate size ranges from 0.1 to 10 mm and is courser than fly ash (Jayaranjan et al., 2014). Bottom ash is mostly silicate, carbonate, aluminate, and ferrous materials (Jayaranjan et al., 2014). Boiler slag is also collected from the bottom of the boiler and is formed under very high temperatures that exceed the fusion temperature and cause the coal to melt (Sajwan et al., 2006). Like bottom ash, boiler slag is granular and abrasive. Bottom ash and boiler slag properties vary depending on the type of coal burned. Both products are applied to roads to improve traction during snow and ice events (Sajwan et al., 2006).

Flue gas desulfurization (FGD) residues are formed by the flue-gas scrubbing systems retrofitted to power plants following the Clean Air Act Amendments of 1990 in order to limit the release of SO_x. This new waste product is formed when sulfur oxides are removed from flue-gases (Sajwan et al., 2006). Flue gas waste is typically a combination of $CaSO_3$ and $CaSO_4$, as well as the sorbent used (typically lime) and

fly ash particles (Sajwan et al., 2006). FGD compositions are constantly changing as plants become more efficient at removing sulfur oxides. Increased scrubbing efficiency increases the sulfur content of the waste product (Sajwan et al., 2006). Prior to storage in a landfill, FGD residues are typically stabilized by mixed with fly ash and quicklime in order to become more stable (Sajwan et al., 2006).

Many factors go into the production of CCWs. Power plants vary in the type of coal burned as well as burning temperatures. The design and technology employed by the power plant and the storage site will affect the characteristics of CCWs, and the mixture of different CCWs makes it difficult to complete risk assessments and manage sites (Asokan et al., 2004). There are commonalities between contaminants expected at each site although toxicity and impacts are site specific.

Properties of Coal Ash

Contamination of the environment surrounding CCW storage sites and how it relates to human and ecosystem health is a topic of significant concern. High concentrations of heavy metals, as well as polycyclic aromatic hydrocarbons (PAHs), and highly acidic leachates can affect local fish populations (Theis & Gardner, 1990). Coal ash effluents have been linked with sublethal effects on behavior, development, and physiology of aquatic organisms (Lohner et al., 2001). Swollen and damaged gill lamellae, tumors, poor egg quality and survival, as well as skeletal malformations and changes in swimming performance have been noted in the biota of habitats surrounding CCW sites (Lohner et al., 2001). Metals and metalloids, particularly copper, lead, selenium, mercury, arsenic, and zinc are present in CCWs and are the major contaminants of concern related to toxicity (Greeley et al., 2014; Lohner et al.,

2001). The complex mixtures of a number of these metals and other trace elements may have synergistic or antagonistic effects on ecosystem health (Heyes et al., 2014). Bioavailability can be decreased when two or more metals interact and bind, thus decreasing toxicity (Heyes et al., 2014). The concentrations of different metals and metalloids in the water column and sediment, and their bioavailability will ultimately determine the toxicological effects these compounds have on the biota.

Coal Ash Contaminants in the Aquatic Environment

Selenium

Selenium is a common contaminant associated with coal ash disposal and is often found to be enriched in ecosystems surrounding CCW storage facilities at or above EPA threshold levels (Rowe et al., 2002). Selenium is an essential micronutrient for many aquatic species in trace amounts but becomes toxic as concentrations increase (Besser et al., 1996). Selenium is cycled between the water column, the sediment, and the biota of aquatic ecosystems. It is taken up by primary producers and microbes and either conserved or concentrated through trophic transfer (Luoma & Presser, 2009). The assimilation and retention of Se within an aquatic environment is dependent on the species and the physical and chemical properties of the environment. The variability in assimilation accounts for the poor linkage between dissolved Se concentrations and bioaccumulation and toxicity (Stewart et al., 2010) Selenium is typically ingested through diet and accumulates in metabolically active tissues, such as the gonads, liver, and kidney, which makes it a threat to early life stages of fish and wildlife. It is linked to adverse effects on reproduction and

development effects and can cause long-term population effects even after the release of coal ash effluent releases is discontinued (Rowe et al., 2002).

An in-situ study of a lake receiving coal ash effluents in Pigeon, Michigan found that Se was bioaccumulating in the fish community at concentrations that could pose a risk to piscivorous birds and mammals (Kennedy et al., 2003). Fish communities in Lake Sutton, North Carolina saw similar effects and contained selenium tissue-concentrations that exceeded toxic thresholds with a high proportion of sunfish (*Lepomis spp.)* demonstrating spinal malformations and deformities (Lemly, 2014). Larval deformities were also reported to result from elevated Selenium concentrations in bluegill (*Lepomis macrochirus)* once concentrations reached 5.9 µg/g wet weight in ovaries (Woock et al., 1987). Se has the ability to interact with a number of other CCW associated trace elements, particularly Hg (Heyes et al., 2014). The interaction of Se and Hg is complex, inconsistent, and concentration and species dependent. Studies have shown positive, negative, and no correlation between Se and Hg interactions (Stewart et al., 2010). Selenium has been shown to protect mammals from Hg toxicity in mammals and fish (Augier et al., 1993; Glynn et al., 1993; Schlenk et al., 2003). Starfish (*Asterias rubens)* exposed to Se and Hg simultaneously accumulated more Se and Hg in tube feet and body wall than starfish exposed to each element alone, suggesting a synergistic interaction (Sørensen & Bjerregaard, 1991). Marine mussels (*Mytilis edulis)* accumulate more Se in the presence of Hg, but Hg accumulation remains the same among exposures to different Se species (Pelletier, 1986). Investigating and understanding the complex

relationships between Se and other trace metals associated with CCW is important because of the potential for biomagnification and population-level impacts.

Arsenic

Like Se, As is another coal ash constituent notable for its high toxicity. The leachability of As depends on the pH and is contingent upon the source coal. Bituminous coal leaches more As than sub-bituminous coal (Pelletier, 1986). Calcium precipitation decreased the leachability of As (Wang et al., 2009). Arsenic can have grave effects on fish but is dependent on environmental conditions (Otter et al., 2012). At sublethal concentrations it can affect immune responses in the walking catfish (*Clarias batrachus)* and antioxidant responses in zebrafish (*Danio rerio)* as well as reproductive and developmental effects in catfish (*Pangasiandon hypothalus),* zebrafish, and mummichog (*Fundulus heteroclitus)* (Otter et al., 2012). Like Se, As also has a tendency to bioaccumulate (Greeley et al., 2016). Arsenic can cause reproductive and developmental impairment and has the potential to impact the vascular system in zebrafish (Greeley et al., 2016). The toxicity of As is dependent on concentration and speciation. Inorgnaic As species are more toxic to aquatic life than organic As species (Meharg & Hartley-Whitaker, 2002). Arsenic concentrations are typically higher in freshwater systems than marine systems because of anthropogenic influences and mine effluents (Bright et al., 1994; Bright et al., 1996). Because of this, freshwater food webs have the potential to bioaccumulate more As compared to marine food webs (Azizur Rahman et al., 2012). Burger et al. (2002) investigated arsenic bioaccumulation in fish of different trophic levels in the Savannah River and found that concentrations averaged from 0.03 to 0.32 µg/g wt weight. Concentrations

were higher in bowfin (*Amina calva)*, primary consumers, than they were in the spotted sucker (*Minytrema melanops)*, a top predator. The accumulation, retention, and transformation process of As within a freshwater environment is therefore complex and may be due to spatial and seasonal variability (Azizur Rahman et al., 2012).

Other Metals

Several studies looking at the effects of CCR mixtures on aquatic organisms have observed greater accumulation of Se and As relative to other trace elements. Hopkins et al. (1999) compared trace element concentrations in banded water snakes and observed Se and As tissue concentrations higher than ever previously reported (1999). Snakes in this study also exhibited Cd levels up to four times higher than concentrations found in reference sites. This study confirmed that unlike As and Se, Cd concentrations can accumulate but do not appear to biomagnify (Hopkins et al., 1999; Wren et al., 1995). Cadmium is not very mobile, although the leaching of Cd increases in fly ash with high chloride content due to complexation (Jones, 1995).

Early life stages of aquatic organisms are particularly sensitive to toxic effects of pollutants. Metal exposed fish are found to contain increased metal tissue concentrations in the gonads (Cd, Cu, Pb, Zn) (Jezierska et al., 2009). Metal exposure can affect fertility and development, as well as spermatozoa motility. Heavy metal concentrations affect a number of metabolic processes in developing fish embryos, and can slow development, increase abnormalities, and decrease growth (Jezierska et al., 2009).

Organics

Studies investigating the toxicity, accumulation, and other effects of CCW have generally focused on inorganic compounds and trace elements, with organic constituents receiving much less attention. Although coal ash is mostly comprised of inorganic constituents, measurable concentrations of dioxins, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) have been observed (Eiceman et al., 1979; Gohda et al., 1993; Ribeiro et al., 2014; Ruwei et al., 2013; Sahu et al., 2009). The presence and concentrations of organic constituents within coal ash are highly variable and dependent on the carbon to hydrogen (C/H) ratio in the coal, as well as the combustion conditions such as temperature, residence time, turbulence, and air to fuel ratio (Sahu et al., 2009). Ruwei et al (2013) completed a study identifying PAHs in fly and bottom ashes and determined concentrations of carcinogenic PAHs that exceeded levels of concern. Concentrations of PAHs within ash were linked to particle size; bottom ash was found to have higher concentrations of PAHs, likely due to the presence of unburned carbon (Ruwei et al., 2013). Further investigation into the bioavailability of PAHs in habits surrounding CCW sites is required (Rowe, 2014).

Major Ion Toxicity

The leachability, fate, and toxicity of As, Cr, Pb, and Se are the primary focus of most CCW studies because of their notable toxicity, although other trace elements and compounds may be enriched in proximity to the facilities and may approach toxic thresholds. Coal ash discharge into Leading Creek Watershed in Meigs County, Ohio was characterized by low in metals, high conductivities, high total dissolved solids

(TDS), and elevated concentrations of major ions (Kennedy et al., 2003; Kennedy et al., 2005). Sulfate (3,672 mg/L), Na (1,952 mg/L), Cl (792 mg/L) and Ca (238 mg/L) were increased due to wastewater treatment and the application of basic reagents in order to neutralize effluent pH (Kennedy et al., 2003). The high concentrations of TDS were linked to in-stream biotic impairment, as measured by in situ toxicity testing measuring clam (*Corbicula fluminea)* growth (Kennedy et al., 2003). Further investigation using a synthetic effluent approach determined that the majority of the acute toxicity of the effluent was determined by Na and SO_4 concentrations (Kennedy et al., 2005).

Major ion toxicity is one of the classes of toxicants that remains difficult to identify, although it is one of the most prevalent causes of toxicity among industrial effluents (Ankley et al., 2011). Initial indicators of ion imbalance as a potential source of whole effluent toxicity consider the salinity or conductivity of the wastewater, the species sensitivity to the effluent, and the failure of the U.S. EPA Phase I TIE manipulations to reduce toxicity (Ankley et al., 2011). Therefore, in the case of suspected major ion toxicity, previous methods have utilized tiered approaches and the creation of synthetic effluents prepared to mimic ionic composition of the effluent (Erickson et al., 2017; Goodfellow et al., 2000; Kunz et al., 2013; McCulloch et al., 1993; Mount et al., 1997). The principal of this approach is to compare toxicity between the synthetic effluent and the actual effluent. Other approaches may involve species sensitivity testing and comparing organisms less sensitive to TDS. *Ceriodaphnia dubia* and *Daphnia pulex* have similar acute sensitives to sodium chloride, whereas *Daphnia magna* is much more tolerant. Fathead minnows

(Pimephales promelas) are the most tolerant (Goodfellow et al., 2000). If a toxic effluent is more toxic to fathead minnows than to *Ceriodaphnia dubia,* sodium chloride could be ruled out as the agent of toxicity.

Mount et al. 1997 developed a multivariate regression model to predict the toxicity attributed to major ion imbalances on three species: *Ceriodaphnia dubia, Daphnia magna,* and fathead minnow (*Pimephales promelas).* The model is helpful as a predictive tool, but it is limited in its application. The model does not consider the influence of diluent water on major ion toxicity, nor does it address complex interactions or sublethal endpoints. The development of the model, as well as previous studies, have focused on acute toxicity. The objective of the present study is to evaluate the chronic toxicity associated with elevated major ions and the respective reductions in reproduction to *C. dubia.*

Project Overview: Summary and Objectives

The purpose of this experiment was to investigate contaminants associated with coal ash disposal and the potential negative impacts on fathead minnow survival and growth as well as *Ceriodaphnia dubia* survival and reproduction. Treated pond effluents from three Maryland coal ash storage facilities were investigated and the toxicities associated with each were determined. It was hypothesized that field-collected media that is found to be toxic will have comparable conductivities and similar levels of trace metals and contaminants.

The first objective was to define the range and variability of toxicity in post-treatment discharge ponds from three Maryland Coal Ash Storage facilities through laboratory-based testing in order to determine the treatment effectiveness and the

potential risk to surrounding ecosystems. Seven-day static renewal bioassays were completed using fathead minnows (*Pimephales promelas)* and *Ceriodaphnia dubia.* Fathead minnows are commonly used as an aquatic toxicity indicator species. They are therefore relatively easy to acquire and threshold toxicities of many contaminants are known and cited in the literature (Ankley & Villeneuve, 2006).

The second objective was to evaluate the toxicity associated with major ion imbalances in CCW leachate by using a mock effluent representative of discharge pond water that reduced *Ceriodaphnia dubia* reproduction. A weight-of-evidence approach was applied in order to determine the primary causative ions. A tiered method was completed in which the total mock effluent was compared to subsequent toxicity tests targeting a single major ion paired with a non-suspect ion in order to investigate how single ions contribute to overall toxicity. It was hypothesized that toxicity at the facilities will vary in relationship to the measured major ions and that the discharge effluent toxicity would be similar to toxicity caused by major ions alone as seen in the mock effluent.

Chapter 2 : Quarterly Testing of Coal Combustion Post-Treatment Leachate Discharge Ponds

Abstract

Dry storage of coal combustion wastes (CCW) can negatively impact the surrounding environment. CCW leachates are characterized by low pH and high conductivity and are often enriched with metals and metalloids that threaten nearby freshwater ecosystems. In the present study, a quarterly series of whole effluent toxicity (WET) tests were completed on facility-treated discharge samples from three Maryland CCW disposal facilities. Priority metals and major ions were measured and monitored over time and concurrently with bioassays. Chronic toxicity was identified at all three locations at varying frequencies, with decreases in *Ceriodaphnia dubia* reproduction as well as decreases in fathead minnow (*Pimephales promelas)* biomass often observed. The wastewaters were consistently high in total dissolved solids (TDS) with high conductivities, evidence that major ion toxicity could be contributing to the effects. Results indicate that the primary causes of toxicity differ between the three facilities, suggesting that site-specific solutions and refinements are required.

Introduction

In 2015 the United States produced 117.3 million tons of coal combustion waste (CCW), or coal ash (American Coal Ash Association, 2017). Beneficial use accounted for 61% of this waste and the remainder was stored in waste facilities. A 2010 report estimated that 300 landfills and 584 storage ponds exist throughout the United States (Zierold & Sears, 2015). These facilities vary in size and construction,

and can be unlined, clay-lined, or composite lined (USEPA, 2010). Coal ash related contaminants can runoff and seep from unlined landfills into the proximate environment. Leachates generated are high in trace elements and can elevate contaminant levels in groundwater, surface water, and soil (Baba et al., 2008). Many of the major coal ash constituents are carcinogenic, neurotoxic, and mutagenic (Chakraborty & Mukherjee, 2009).

Contamination of the environment surrounding CCW storage sites and its relation to human and ecosystem health is a topic of significant concern. High concentrations of heavy metals, as well as polycyclic aromatic hydrocarbons (PAHs), and highly acidic leachates can affect local fish populations (Theis & Gardner, 1990). Coal ash effluents have been linked with sublethal effects on behavior, development, and physiology of aquatic organisms (Lohner et al., 2001). Swollen and damaged gill lamellae, tumors, poor egg quality and survival, as well as skeletal malformations and changes in swimming performance have been noted in the biota of habitats surrounding CCW sites (Lohner et al., 2001). Metals and metalloids, particularly copper, lead, selenium, mercury, arsenic, and zinc, are present in CCWs and are the major contaminants of concern related to toxicity (Greeley et al., 2014; Lohner et al., 2001). The complex mixtures of these metals and other trace elements may have synergistic or antagonistic effects on ecosystem health (Heyes et al., 2014). Bioavailability, and thus toxicity, can be decreased when two or more metals interact and bind (Heyes et al., 2014). The concentrations of metals and metalloids in the water column and the sediment, and their bioavailability will contribute to the toxicological effects these compounds have on the biota.

The present study investigated the toxicity of post-treatment pond effluents surrounding three Maryland coal ash storage facilities. These are dry storage facilities in which fly ash, bottom ash, and gypsum generated both on and off-site are stored in landfill cells, called monofills. These facilities were constructed in the 1970s-1980s and include currently operational cells as well as historic cells that have been capped with a soil layer and vegetation. They are unlined, allowing leaching ach via groundwater migration. Leachates are pumped and treated with a series of active treatment systems and then held in a pond. The treated pond water is discharged as needed within each facility and associated with a National Pollutant Discharge Elimination System (NPDES) permit. NPDES permits, required under the Clean Water Act (CWA), regulate maximum contaminate levels in effluents and can require WET testing. The three facilities are within the Chesapeake Bay Watershed and are managed by NRG Energy Inc.: Brandywine, located in Prince George's County and adjacent to the Mattaponi Creek which connects to the Patuxent River; Faulkner, located in Charles County and proximal to the Zekiah Swamp; and Westland, located in Montgomery Country and neighboring the Potomac River (Figure 2.1). Lethal and sublethal effects of the effluents on laboratory species were examined quarterly through short-term chronic whole effluent toxicity (WET) testing using 7-day static renewal bioassays (USEPA, 2002). The results of the toxicity tests were compared to reported concentrations of metals, major ions, and water quality.

Methods

Sample Collection

Grab samples from the 3 facilities (Figure 2.3) were collected in three replicates of 10 L high-density polyethylene (HDPE) bottles with minimal headspace, put on ice at collection, and refrigerated upon arrival. Collection dates are presented in Table 2.1. Each quarter will henceforth be referred to as "Q1, Q2, Q3, and Q4." Maryland biomonitoring program requires that if any two consecutive valid toxicity tests demonstrate acute or chronic toxicity an additional test must be repeated within 30 days in order to confirm the findings (MDE, 2012). 2017 Q3-1 therefore refers to the first test completed in the third quarter of 2017, and 2017 Q3-2 refers to the additional test competed in the third quarter of 2017. Tests were started within 4 hours of collection. Samples were refrigerated and kept in the dark throughout the 7-day test.

Table 2.1 Dates of quarterly testing at each facility; 2018 Q3-2 denotes the second quarterly test done on a second sample collected during the 3rd quarter of 2017, per MDE requirements

Toxicity Test Dates			
	Brandywine	**Faulkner**	**Westland**
2017 Q2	6/6/17	5/19/17	6/22/17
2017 Q3-1	8/24/17	8/16/17	8/24/17
2017 Q 3-2	9/27/17		
2017 Q4	12/13/17	12/13/17	12/5/17
2018 Q1	2/21/18	2/21/18	2/28/18
2018 Q2	6/12/18	6/12/18	6/26/18
2018 Q3	9/12/18	9/12/18	9/20/18

Site Locations

The Brandywine Ash Storage Site is located at the intersection of North Keys Road and Gibbons Church Road in the town of Brandywine in Prince George's County, Maryland. The Westland Ash Storage Site is located on Martinsburg Road in Dickerson, Maryland. The Faulkner Ash Storage Site is located at 1260 Crain Highway in Newburg, Maryland (Figure 2.1).

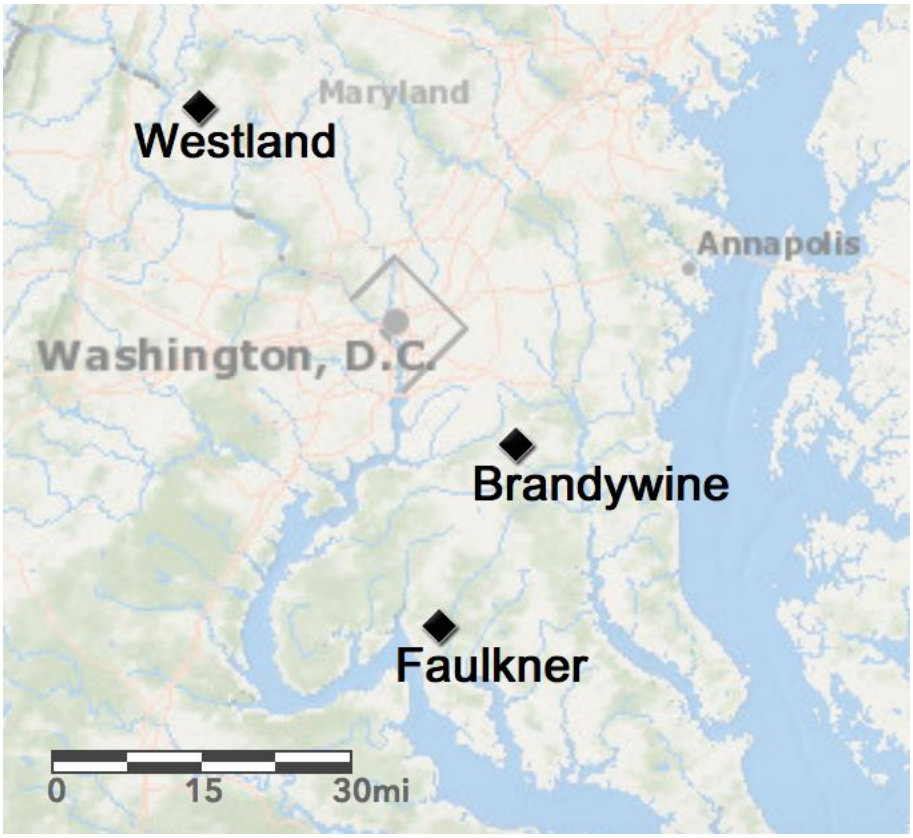

Figure 2.1 Location of 3 facilities

Test Organisms

Larval fathead minnows (*Pimephales promelas)* less than 24 hours old were purchased from Aquatic Biosystems Inc. in Fort Collins, CO. Neonate *Ceriodaphnia dubia* less than 48 hours old and hatched within a 24-hour window were cultured

inhouse in diluted mineral water (DMW) consisting of 20% Perrier™ and 80% distilled water. Initial quarterly testing that took place in 2017 Q2 and Q3 utilized randomized batch-cultured neonates. For the 2017 Q4 and all subsequent tests, brood boards were maintained, and neonates of known parentage were placed in treatments such that each treatment contained 10 to 12 replicate individuals from unique parents, and each treatment was genetically identical to the other treatments.

Laboratory Protocols

Toxicity testing protocols followed EPA Method 1002.0 (USEPA, 2002). *C. dubia* and fathead minnows were exposed to post-treatment pond effluents in a 7-day static renewal system using a dilution series of 10%, 18%, 32%, 56%, and 100%. Tests were maintained at 25º C in a temperature-controlled test chamber with a 16h light/8h dark light cycle. Diluted mineral water was used as control water and diluent. Each day refrigerated effluent samples were vacuum filtered on a 0.7 μm pre-filter via glass fiber filtration (GFF) to remove any native organisms, aerated, and brought to 25º C in a warm-water bath prior to renewal.

Fathead minnow tests were conducted using four replicate test chambers of each treatment with 10 fish in each replicate (Figure 2.2). Glass test chambers of 400 mL volume were filled with 300 mL of test solution at the start of the test and received 90% volume replacement daily by siphoning and renewal. Fathead minnows were fed <72-hour old *Aretemia* nauplii twice daily.

C. dubia tests were conducted using 10 to 12 test chambers at each treatment with one neonate in each chamber at the start of the test (Figure 2.3). Leached polystyrene chambers of 30 mL volumes were filled with 15 mLs of test solution.

Each day, a new set of chambers was prepared, and organisms were transferred from original test chambers into new chambers. Old test chambers were held overnight so that young could be counted after 24-hours had passed and young were more visible. Toxicity testing protocols followed EPA Method 1000.0 (USEPA 2002). *C. dubia* were fed a 250 µL aliquot of a 1:1 mixture of algae (*Raphidocelis subcapitata)* 6.0 x 10^7 cells/ml and yeast-Cerophyll-trout chow (YCT) daily at each renewal.

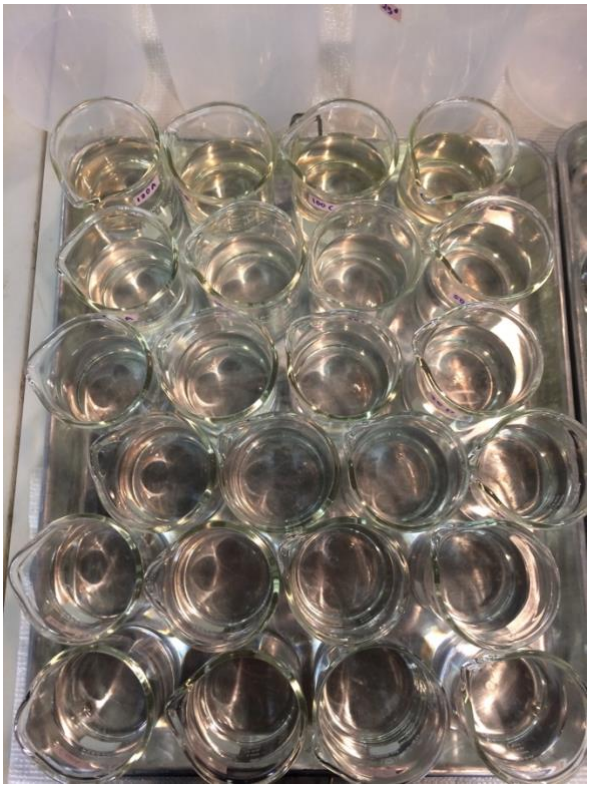

Figure 2.2 Fathead minnow test chambers

Figure 2.3 *C. dubia* test chambers

Water Quality

Temperature, pH, dissolved oxygen (DO), conductivity, alkalinity, hardness, and ammonia were measured at the start of each test (day 0) for both the 100% effluent and the control water treatments. Temperature, pH, and DO were measured for the renewal water prepared daily for each treatment, as well as the 24-hour old samples taken from test chambers prior to renewal.

Chemical Analysis

Daily, weekly, and monthly analysis of metals, major ions, and water quality characteristics of the post-treatment discharge ponds was completed by Microbac

Laboratories Inc. in Baltimore, Maryland (Table 2.2). Analytical methods generally comprised of inductively coupled plasma mass spectrometry (ICP/MS), see appendix for complete list of methods.

Table 2.2 Frequency of analysis for each analyte corresponding to the frequency at which the treated effluent pond was discharged

Frequency of Analysis Upon Discharge	Analyte / Parameter
Daily	Arsenic, cadmium, calcium, chloride, copper, lead, magnesium, nickel, selenium, zinc, hardness
Weekly	Conductivity, dissolved oxygen, sulfate, temperature, total dissolved solids (TDS)
Monthly	Boron, chromium, iron

Endpoints and Statistics

Lethality and growth as indicated by biomass served as toxicological endpoints for the fathead minnow. Lethality and reproduction as indicated by total neonate production served as toxicological endpoints for *C. dubia.* No observable effect concentrations (NOECs) were calculated using Analysis of Variance (ANOVA). Fathead minnow biomass was calculated by the dry weight per number of original organisms in each replicate and was selected as a more sensitive sublethal endpoint. Inhibition Concentration 25s (IC25) were calculated using linear interpolation methods and were used to compare toxicity between tests. All analysis was completed using the USEPA WET Analysis Spreadsheet v1.6.1 (https://www3.epa.gov/npdes/pubs/wet_analyticalspreadsheet.xls).

No observable effect concentrations are limited to selected treatment concentrations; therefore, the intervals are determined by the selected treatment

concentrations. Independence of each test chamber was assumed. Normal data and homogeneity of variance were tested using Shapiro-Wilk test and Bartlett's test, respectively (USEPA, 2002). Tests with normally distributed data that passed homogeneity of variance with equal number of replicates were tested using Dunnett's test (USEPA, 2002). Tests with unequal number of replicates were tested using a t-test with Bonferroni adjustment. Tests with non-normally distributed data and/or heterogeneity of variance with equal number of replicates were tested using Steel's Rank Test, tests with unequal replicates were tested using Wilcoxon Rank Sum Test with Bonferroni adjustment (USEPA, 2002). Alpha levels of 0.01 were used for testing the assumptions of parametric analysis. Alpha levels of 0.05 were used to determine NOECs.

Point-estimation techniques were used to calculate IC25s. The linear interpolation method assumes responses are non-increasing, follow a linear-response function, and are from independent and representative data (USEPA, 2002). In cases in which the means of each treatment increased, these data were adjusted by smoothing. The precision of these estimates within each test was assessed by the 95% confidence intervals calculated by the bootstrap method (USEPA, 2002). Precision among tests was compared by standard deviations.

Differences in yearly measured water quality parameters and analytes (when available) were compared using a two-tailed t-test.

Results and Discussion

Quarterly Toxicity Testing

Water Quality

Water quality parameters (pH, DO, ammonia, alkalinity) fell within normal ranges. Hardness could not be determined using ethylenediaminetetraacetic acid (EDTA) titration. Sample water quality data is provided in appendix A.

Brandywine

The Brandywine treated effluent was toxic to *C. dubia* six out of six quarters and the most common effect was a reduction in reproduction (Table 2.3a). IC25 values ranged from 32.5 to 72.3%. Reductions in survival to *C. dubia* were significant on two occasions, 2017 Q2 and 2017 Q4. Fathead minnow biomass was reduced 6 out of 7 quarters with IC25s < 100% (Table 2.3b). The 2018 Q1 sample had a NOEC of 56% but an IC25 >100%. In this case, the 100% treatment was reduced relative to the control, but not reduced by 25%, which caused the concentration at which a 25% reduction is expected to be calculated as >100%. The 2017 Q3-1 sample had a NOEC of 100% but an IC25 of 90.3%. This was due to the large percent minimal statistical difference (PMSD) and the differences in calculating NOECs and IC25s. In all cases, the IC25 for *C. dubia* reproduction was lower than the IC25 for the fathead minnow biomass, with the exception of the 2018 Q2 sample, which was slightly more toxic to the fathead minnow. This suggests that *C. dubia* are typically more sensitive to the constituents causing toxicity in the treated effluent at Brandywine, and that the 2018 Q2 sample was potentially unique in its makeup.

Table 2.3a Quarterly Toxicity Test Results Brandywine (*C. dubia*)

	% Survival		Reproduction						
Quarter	**Control**	**NOEC**	**Control**	**NOEC**	**PMSD**	**TU**	**IC25**	**LOWER LIMIT**	**UPPER LIMIT**
2017 Q2	90	**56**	23.8	**56**	34	**1.66**	**60.2**	42.5	65.2
2017 Q3-1	100	100	23.0	**56**	22.5	**1.70**	**58.7**	47.8	64.4
2017 Q3-2*	83.3	100	26.8	**32**	22.5	**2.22**	**45.1**	38.5	50.7
2017 Q4	100	100	19.6	**56**	61.6	**2.20**	**45.5**	25	59.1
2017 Q4**	100	**56**	28.0	**32**	26.8	**2.58**	**38.7**	37.8	40.4
2018 Q1	100	100	32.8	**32**	9.4	**1.38**	**72.3**	59.4	79.8
2018 Q2	90	100	33.1	**32**	26.7	**3.08**	**32.5**	7.0	40.3
2018 Q3	100	100	25.3	**32**	22.1	**2.46**	**40.6**	39.2	42.2
MEAN	95.4	89.0	26.6	41.0	28.2	**2.2**	**49.2**	37.2	55.3
SD	6.66	20.37	4.69	12.42	15.16	**0.56**	**13.30**	15.58	14.32

* denotes the second quarterly test done on a second sample collected during the 3rd quarter of 2017, per MDE requirements

**denotes the retesting of the 12/13/17 sample in which control reproduction was not within an acceptable range

Table 2.3b Quarterly Toxicity Test Results Brandywine (Fathead Minnow)

	% Survival		Growth (biomass)						
Quarter	**Control**	**NOEC**	**Control**	**NOEC**	**PMSD**	**TU**	**IC25**	**LOWER LIMIT**	**UPPER LIMIT**
2017 Q2	95	100	0.48	**56**	17.0	**1.36**	**73.3**	66.8	79
2017 Q3-1	100	100	0.54	100	20.8	**1.11**	**90.3**	72.3	95.1
2017 Q3-2*	92.5	**56**	0.58	**56**	14.7	**1.66**	**60.1**	56	62.4
2017 Q4	97.5	100	0.26	**56**	21.9	**1.39**	**72.1**	68.6	73.1
2018 Q1	100	100	0.50	**56**	9.7	<1.0	>100	na	na
2018 Q2	95	**56**	0.45	**32**	21.7	**3.05**	**32.8**	7.0	59.8
2018 Q3	100	**56**	0.51	**32**	10.0	**2.32**	**43.1**	40.2	44.8
MEAN	97.1	81.1	0.5	55.4	16.5	**1.82**	**62.0**	51.8	69.0
SD	3.04	23.52	0.10	22.68	5.27	**0.73**	**21.19**	24.85	17.39

* denotes the second quarterly test done on a second sample collected during the 3rd quarter of 2017, per MDE requirements

Faulkner

The Faulkner treated effluent reduced *C. dubia* survival and reproduction on one occasion (2017 Q4) on this occasion the survival NOEC was 56% and the IC25

was 73.5% (Table 2.4). The 2018 Q3 sample reduced fathead minnow survival and growth but did not significantly impact *C. dubia* reproduction (Table 2.4b). In contrast to Faulkner, *C. dubia* were typically more sensitive than fathead minnows to the Brandywine treated effluent.

Table 2.4a Quarterly Toxicity Test Results Faulkner (*C. dubia*)

	% Survival		Reproduction						
Quarter	**Control**	**NOEC**	**Control**	**NOEC**	**PMSD**	**TU**	**IC25**	**LOWER LIMIT**	**UPPER LIMIT**
2017 Q2	92	100	15.2	100	?	<1.0	>100	na	na
2017 Q3	100	100	18.1	100	?	<1.0	>100	na	na
2017 Q4	100	**56**	36.9	**56**	15.9	**1.36**	**73.5**	60.3	88.8
2018 Q1	92	100	28.9	100	24.8	<1.0	>100	na	na
2018 Q2	90	100	31.3	100	31	<1.0	>100	na	na
2018 Q3	100	100	28.9	100	28.8	<1.0	>100	na	na
MEAN	95.7	92.7	26.6	92.7	25.1				
SD	4.80	17.96	8.26	17.96	6.66				

Table 2.4b Quarterly Toxicity Test Results Faulkner (Fathead Minnow)

	% Survival		Growth (biomass)						
Date	**Control**	**NOEC**	**Control**	**NOEC**	**PMSD**	**TU**	**IC25**	**LOWER LIMIT**	**UPPER LIMIT**
2017 Q2	97.5	100	0.56	100	?	<1.0	>100	na	na
2017 Q3	100	100	0.55	100	?	<1.0	>100	na	na
2017 Q4	100	100	0.31	100	20.3	<1.0	>100	na	na
2018 Q1	95	100	0.42	100	15.3	<1.0	>100	na	na
2018 Q2	92.5	100	0.39	100	19.7	<1.0	>100	na	na
2018 Q3	97.5	**56**	0.5	**56**	16.7	**1.26**	79.1	62.7	98.4
MEAN	97.1	92.7	0.47	92.7	18.0				
SD	2.92	17.96	0.10	17.96	2.39				

Westland

The Westland treated effluent reduced reproduction in *C. dubia* three out of seven times it was tested (Table 2.5a). The Westland effluent did not significantly reduce the survival of *C. dubia* at any time point for sampling. The 2018 Q1 sample was determined to have an IC25 of 88% and a NOEC of 100%. This is due to the

differences in calculating NOECs and IC25s. The Westland treated effluent did not reduce growth or survival for the fathead minnow (Table 2.5b).

Table 2.5a Quarterly Toxicity Test Results Westland (*C. dubia*)

	% Survival		Reproduction						
Date	**Control**	**NOEC**	**Control**	**NOEC**	**PMSD**	**TU**	**IC25**	**LOWER LIMIT**	**UPPER LIMIT**
2017 Q2	100	100	38.3	100	?	<1.0	>100	na	na
2017 Q3	100	100	23.3	100	?	<1.0	>100	na	na
2017 Q4	100	100	20.8	**56**	18.3	**1.56**	**63.9**	59.5	66.7
2018 Q1	100	100	32.1	100	19.1	**1.14**	**88**	78.1	97.7
2018 Q2	100	100	24.6	**56**	26.7	**1.46**	**68.6**	59.4	70.9
2018 Q3	100	100	35.3	**100**	16.6	<1.0	>100	na	na
MEAN	100	100	29.1	85.3	20.2	1.4	73.5	65.7	78.4
SD	0.00	0.00	7.14	22.72	4.47	0.22	12.78	10.77	16.82

Table 2.5b: Quarterly Toxicity Test Results Westland (Fathead Minnow)

	% Survival		Growth (biomass)						
Date	**Control**	**NOEC**	**Control**	**NOEC**	**PMSD**	**TU**	**IC25**	**LOWER LIMIT**	**UPPER LIMIT**
2017 Q2	97.5	100	0.40	100	?	<1.0	>100	na	na
2017 Q3	100	100	0.49	100	?	<1.0	>100	na	na
2017 Q4	92.5	100	0.47	100	20.3	<1.0	>100	na	na
2018 Q1	100	100	0.42	100	15.3	<1.0	>100	na	na
2018 Q2	92.5	100	0.38	100	22.7	<1.0	>100	na	na
2018 Q3	97.5	100	0.34	100	19.9	<1.0	>100	na	na
MEAN	96.7	100	0.42	100.0	19.6				
SD	3.42	0.00	0.06	0.00	3.09				

Treated Effluent Analyte Concentrations

The Brandywine post-treatment discharge water was low in metals (Table 2.6). Arsenic, Cr, Cu, Fe, Pb, Hg, Ni, Se, and Zn are below water quality criteria (WQC). Boron, cadmium, and chloride exceeded EPA recommended WQCs. Metal concentrations were reduced relative to the literature values for CCW effluents which suggests the treatment system is effective at removing metals. Conductivity changed over time relative to TDS. Hardness was consistently high. High conductivity and

high TDS at Brandywine are consistent with the elevated concentrations of major ions observed. The chloride and sulfate concentrations reported on 2017 Q2 were outliers and possibly reported incorrectly. Critical major ions, including K and Na were not measured during daily/weekly/monthly analysis. The sum of dissolved Ca, Cl, Mg, and SO_4 do not add up to the total dissolved solids because some solids were not analyzed. However, it is important to note that the measurements listed in table 2.6 are for total concentrations, and not dissolved.

Table 2.6: Brandywine daily/weekly/monthly analytes as measured at time of collection; values represent total concentrations, blank cells represent values not reported, colored cells represent analytes that exceeded WQC

	Brandywine				Max	Lit. CCW Values[4]	Water Quality Criteria
	2017 Q2	2017 Q3	2017 Q3-2	2017 Q4			
Arsenic (µg/L)	3.6	<150	1.46	0.82	3.6	58-100 µg/L	150 µg/L[1]
Boron (mg/L)	18				18		1.5 mg/L[2]
Cadmium (µg/L)	0.6	<0.25	0.146	0.28	0.6	100-123 µg/L	0.25 µg/L[3]
Calcium (mg/L)	390	320	490	510	510		
Chloride (mg/L)		720	920	830	920		230 mg/L[2]
Chromium (µg/L)	<0.2					160-200 µg/L	100 µg/L[3]
Copper (µg/L)	4	<9.0	4.4	7.5	7.5	390-660 µg/L	9 µg/L[3]
Iron (mg/L)	<0.20						1 mg/L[1]
Lead (µg/L)	<1	<250	<1	<1		NR	2.5 µg/L[1]
Magnesium (mg/L)	120	79	110	150	150		
Mercury (µg/L)	<0.2						0.77 µg/L[1]
Nickle (µg/L)	14.3	<52	16.7	19	19		52 µg/L[1]
Selenium (µg/L)	0.0121	6.1	3.71	3.84	6.1	100-110 µg/L	5 µg/L[3]
Sulfate (mg/L)		1300	1900	1900	1900		
Zinc (µg/L)	<5	<120	<5	<5			120 µg/L[1]
Hardness (as $CaCO_3$)	1500	1100	1700	1900	1900		
Conductivity (µS/cm)	5260	4710	5800	6470	6470		
TDS (mg/L)	4400	3600	5300	4000	5300		

[1] EPA Water Quality Criteria- Aquatic Life Criteria Table
[2] Canadian Environmental Quality Guidelines
[3] Code of Maryland Regulations (COMAR) 26.08.03.06
[4] Values derived by Rowe et al. 2002 and references therein

Table 2.7 Major ion concentrations and conductivity as measured every time effluent discharge ponds were emptied between May and December 2017 (2017 Q2 to 2017 Q4)

Analyte / Parameter	Brandywine 2017		Brandywine 2018		Faulkner 2017		Westland 2017	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Calcium (mg/L)	467	109	375	79	277	29	281	84
Chloride (mg/L)	942	264	664	143	410	85	199	68
Magnesium (mg/L)	121	24	89	21	98	11	60	19
Sulfate (mg/L)	1840	349	1534	333	1171	142	785	131
Conductivity (µs/cm2)	5585	670	4574	983	3100	443	1982	366

The 2017 Q4 Brandywine sample had the highest conductivity and the highest hardness among all samples, however, TDS was lower than the 2017 Q3-2 sample. Magnesium was elevated relative to the Q3-2 sample, sulfate was high but consistent with the 2017 Q3-2 sample. Chloride was lower than the 2017 Q3-2 sample. The relationship between conductivity and the regularly measured major ions (Ca, Cl, Mg, and SO_4) is complex and better shown in Figure 2.4a and Figure 2.4b**.** Figure 2.4a&b illustrates the measured ion concentrations and conductivity following the installation of active treatment systems in May 2017 through December 2017. Magnesium remained at ~100 mg/L throughout the time interval and was very consistent with a standard deviation of 24 mg/L. Calcium was also fairly consistent with a standard deviation of 109 mg/L and spiked only in early September. Chloride had a mean of 942 mg/L, (Table 2.7) and varied during the summer months. Chloride, Ca, and Mg concentrations did not greatly influence conductivity. Sulfate concentrations were the most variable and had the greatest influence on conductivity for the Brandywine effluent. The 2018 Brandywine samples had mean major ion concentrations lower

than those measured in 2017 (Figure 2.4b, Table 2.7). Conductivity was significantly lower (P-value <0.001).

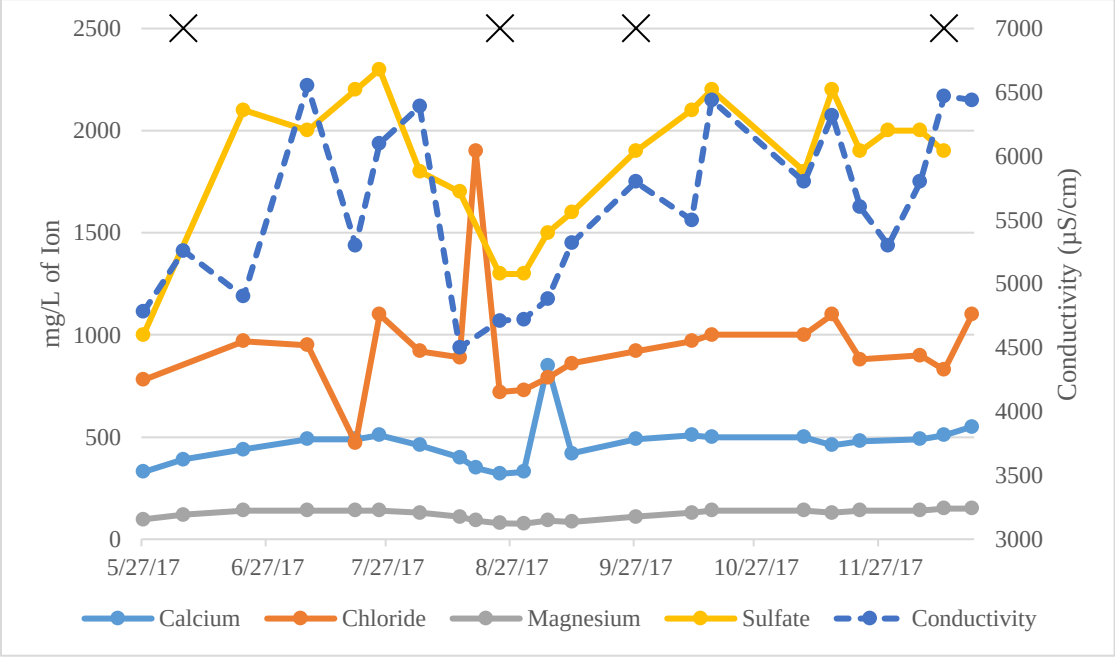

Figure 2.4a Brandywine major ions over time for available data in 2017 (mg/L) as measured every time effluent discharge pond was drained; "X" denotes dates in which toxicity testing occurred

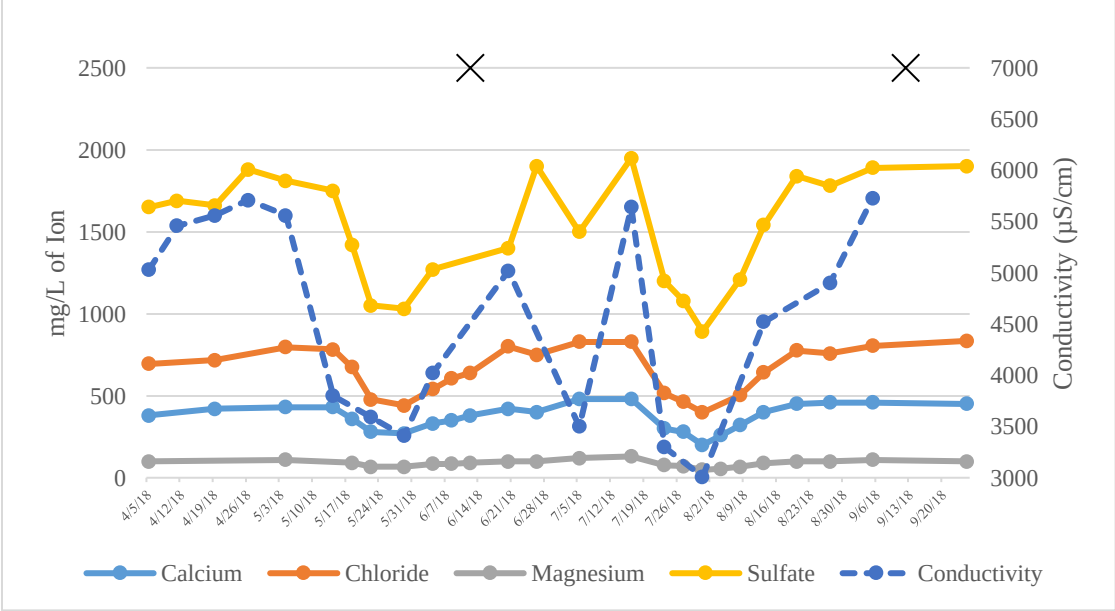

Figure 2.4b Brandywine major ions over time for available data in 2018 (mg/L) as measured every time effluent discharge pond was drained; "X" denotes dates in which toxicity testing occurred

The Faulkner facility post-treatment discharge pond water was less enriched with most constituents at the time of quarterly toxicity testing compared to Brandywine (Table 2.6, 2.7, 2.8**)**. Conductivity and TDS were also lower at Faulkner than at Brandywine. Mean hardness at the time of quarterly testing of the two facilities was similar. Hardness is largely a product of Ca and Mg, although other metals do contribute (O'Shea & Mancy, 1978). Calcium, Cl, Mg, and SO_4 averaged lower than major ions measured at Brandywine. Zinc and Se were both higher at Faulkner than at Brandywine. Zinc was below WQC but Se was slightly higher. Boron and Cl also exceeded WQCs. WQCs are often conservative values, so exceedances do not confirm a cause of toxicity.

Table 2.8 Faulkner daily/weekly/monthly analytes as measured at time of collection; values represent total concentrations, blank cells represent values not reported, colored cells represent analytes that exceeded WQC

	Faulkner			Max	Literature CCW Values[4]	Water Quality Criteria
	2017 Q2	2017 Q3	2017 Q4			
Arsenic (µg/L)	0.0027	5.6	0.78	5.6	58-100 µg/L	150 µg/L[1]
Boron (mg/L)		5.2	5.4	5.4		1.5 mg/L[2]
Cadmium (µg/L)	<0.2	0.11	0.058	0.11	100-123 µg/L	0.25 µg/L[3]
Calcium (mg/L)	240	250	290	290		
Chloride (mg/L)	350	350	380	380		230 mg/L[2]
Chromium (µg/L)		3	<0.2	3	160-200 µg/L	100 µg/L[3]
Copper (µg/L)	2.4	5.0	4.2	5.0	390-660 µg/L	9 µg/L[3]
Iron (mg/L)		<0.20	<0.20			1 mg/L[1]
Lead (µg/L)	<1	<5	<0.06		NR	2.5 µg/L[1]
Magnesium (mg/L)	92	86	110	110		
Mercury (µg/L)		<0.2	<0.2			0.77 µg/L[1]
Nickle (µg/L)	10.8	15.0	13.9	15.0		52 µg/L[1]
Selenium (µg/L)	8.2	9.1	2.78	9.1	100-110 µg/L	5 µg/L[3]
Sulfate (mg/L)		1100	1100	1000		
Zinc (µg/L)	5.5	8.3	<0.9	8.3		120 µg/L[1]
Hardness (as $CaCO_3$)	1000	970	1200	1200		
Conductivity (µS/cm)		2700	3440	3440		
TDS (mg/L)		2500	2600	2600		

[1] EPA Water Quality Criteria- Aquatic Life Criteria Table
[2] Canadian Environmental Quality Guidelines
[3] Code of Maryland Regulations (COMAR) 26.08.03.06
[4] Values derived by Rowe et al. 2002 and references therein

When looking at the relationship of major ions over time, Faulkner appeared to be more consistent than Brandywine (Figure 2.5 vs Figure 2.4). Like Brandywine, SO_4 dominated the wastewater and SO_4 and conductivity tracked closely with one another. Chloride peaked to 730 mg/L in late May. Sulfate increased from 1200 mg/L to 1300 mg/L in late September, although this was not met with a change in

overall conductivity. In mid-July there was a small drop in the concentration of each measured major ion as well as conductivity. This could be due to increased rainfall prior to sampling which could have diluted the pond. Magnesium was relatively consistent overtime and did not fluctuate much, analogous to what is seen at Brandywine.

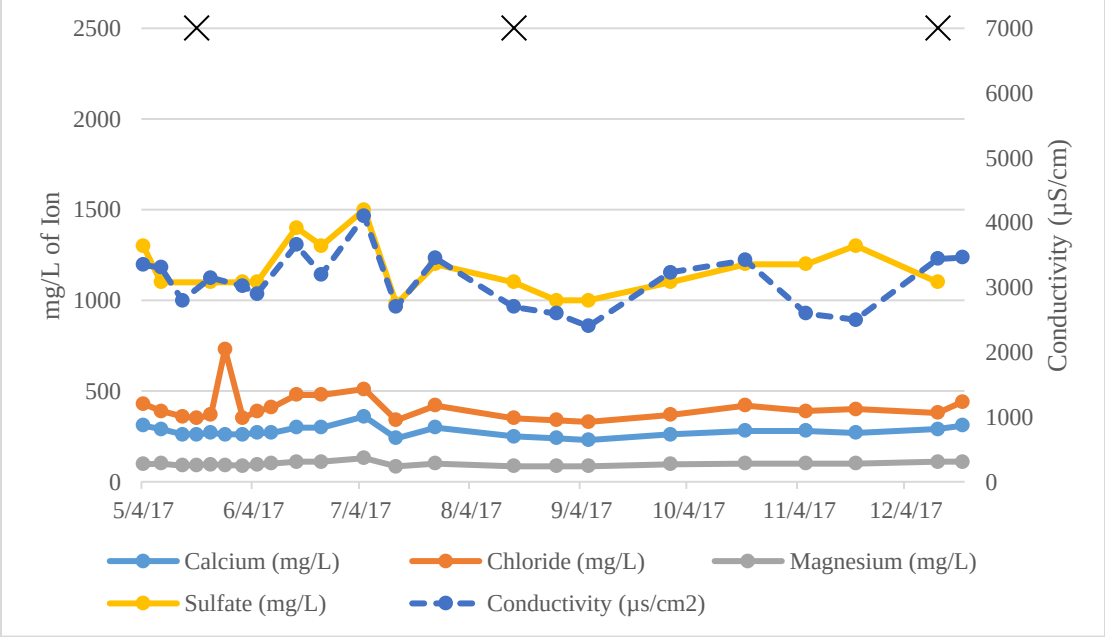

Figure 2.5 Faulkner major ions over time (2017) (mg/L) as measured every time effluent discharge pond was drained; "X" denotes dates in which toxicity testing occurred

With regard to major ions, conductivity, hardness, and TDS, Westland was the least enriched of the three sites (Table 2.9 vs Tables 2.6 & 2.7). Boron levels were higher at Westland than at Faulkner but lower than at Brandywine. Boron levels exceeded recommended WQC. Most metals were all within the WQC. Selenium at Westland was significantly higher than the other two sites and exceeded the WQC. Selenium is notable for its narrow concentration range between what is required for life and what is toxic and it could potentially be a contributor to toxicity.

Table 2.9 Westland daily/weekly/monthly analytes as measured at time of collection; values represent total concentrations, blank cells represent values not reported, colored cells represent analytes that exceeded WQC

	Westland			Max	Literature CCW Values[4]	Water Quality Criteria
	2017 Q2	2017 Q3	2017 Q4			
Arsenic (µg/L)	3.00	<150	0.72	3.00	58-100 µg/L	150 µg/L[1]
Boron (mg/L)	7		13	13		1.5 mg/L[2]
Cadmium (µg/L)	<0.25	<0.250	0.155	0.155	100-123 µg/L	0.25 µg/L[3]
Calcium (mg/L)	210	250	110	250		
Chloride (mg/L)	210	190	250	250		230 mg/L[2]
Chromium (µg/L)	<0.2		<0.2		160-200 µg/L	100 µg/L[3]
Copper (µg/L)	2	<9.0	3.2	3.2	390-660 µg/L	9 µg/L[3]
Iron (mg/L)	<0.20		<0.10			1 mg/L[1]
Lead (µg/L)	<1	<2.50	<1		NR	2.5 µg/L[1]
Magnesium (mg/L)	38	53	70	70		
Mercury (µg/L)	<0.2		<0.2			0.77 µg/L[1]
Nickle (µg/L)	10.2	<52	12.8	12.8		52 µg/L[1]
Selenium (µg/L)	3	13.6	17.7	17.7	100-110 µg/L	5 µg/L[3]
Sulfate (mg/L)	550	690	810	810		
Zinc (µg/L)	<5	<120	<5			120 µg/L[1]
Hardness (as $CaCO_3$)	690	840	1100	1100		
Conductivity (µS/cm)	1600	1600	2450	2450		
TDS (mg/L)	1400	1400	1900	1900		

[1] EPA Water Quality Criteria- Aquatic Life Criteria Table
[2] Canadian Environmental Quality Guidelines
[3] Code of Maryland Regulations (COMAR) 26.08.03.06
[4] Values derived from Rowe et al. 2002 and references therein

Like Brandywine and Faulkner, Westland's effluent is also sulfate-dominated (Figure 2.6). At Westland, Ca tends to be higher than Cl whereas Cl predominates Ca at both Brandywine and Faulkner. Magnesium concentrations are the lowest of the measured major ions at Westland, and at lower concentrations than those measured at

the other two sites. In mid-April there were decreases in Ca, Mg, and SO_4. This could be the result of a recent rainfall diluting ponds.

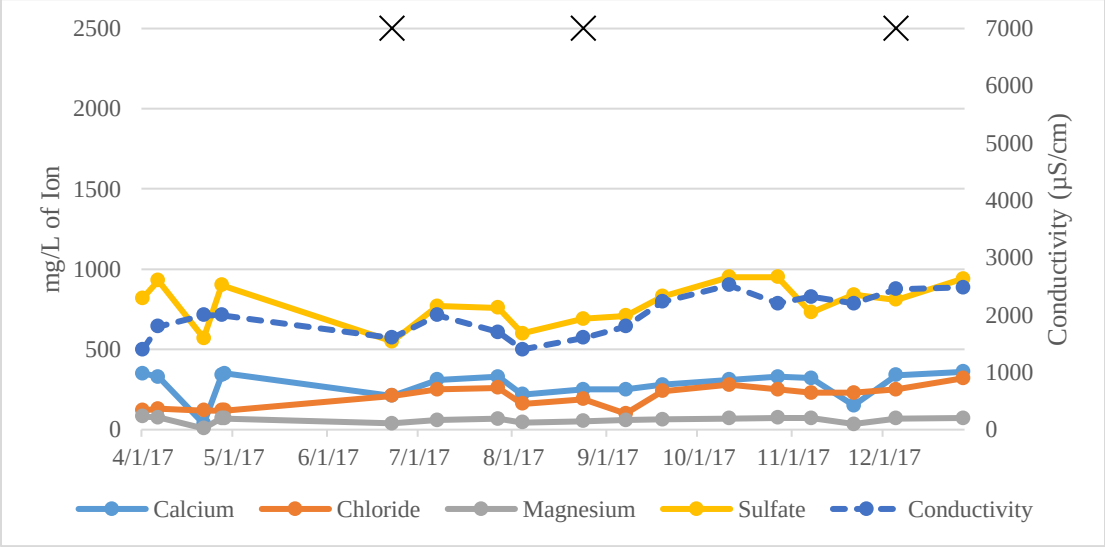

Figure 2.6 Westland major ions over time (2017) (mg/L) as measured every time effluent discharge pond was drained; "X" denotes dates in which toxicity testing occurred

Relationships Between Toxicity and Chemistry

The Brandywine effluent was the most toxic and consistently reduced reproduction to *C. dubia* and biomass to fathead minnows. The Brandywine effluent had the highest conductivity, TDS, and major ion concentrations (for the major ions measured) of the three sites. Increased effluent conductivity roughly correlated with an increased IC25 (R^2 of 0.3235; Figure 2.7). Figure 2.8 shows the relationship between conductivity and percent control biomass for each quarterly test at Brandywine in which a reduction in biomass was observed. A biphasic response is sometimes seen in which low treatment concentrations outperform the control treatments. The red line is an outlier and represents 2017 Q4 in which the lab temperature was out of compliance (20°C) and control growth was poor. Minimum

growth requirements were met but the lower temperature was unusual and may explain the poor control performance.

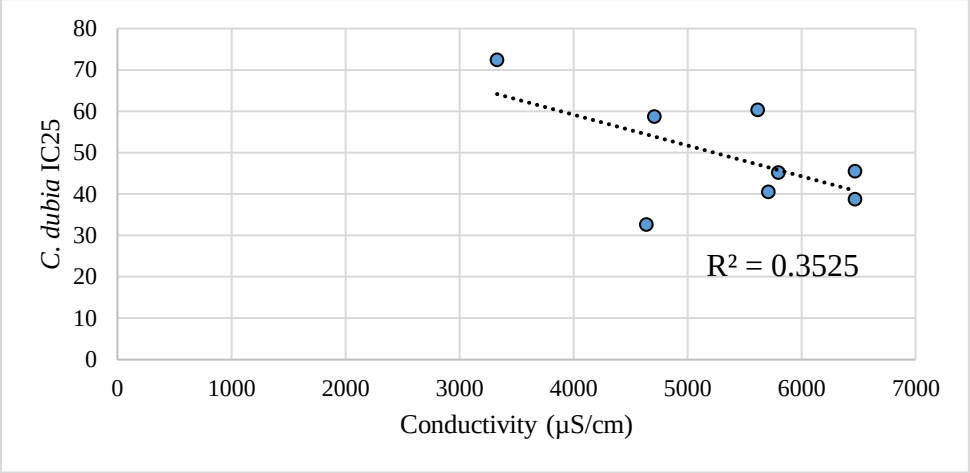

Figure 2.7 Brandywine Coal Ash leachate 7-day *C. dubia* reproduction IC25 as it relates to conductivity for each quarterly discharge sample

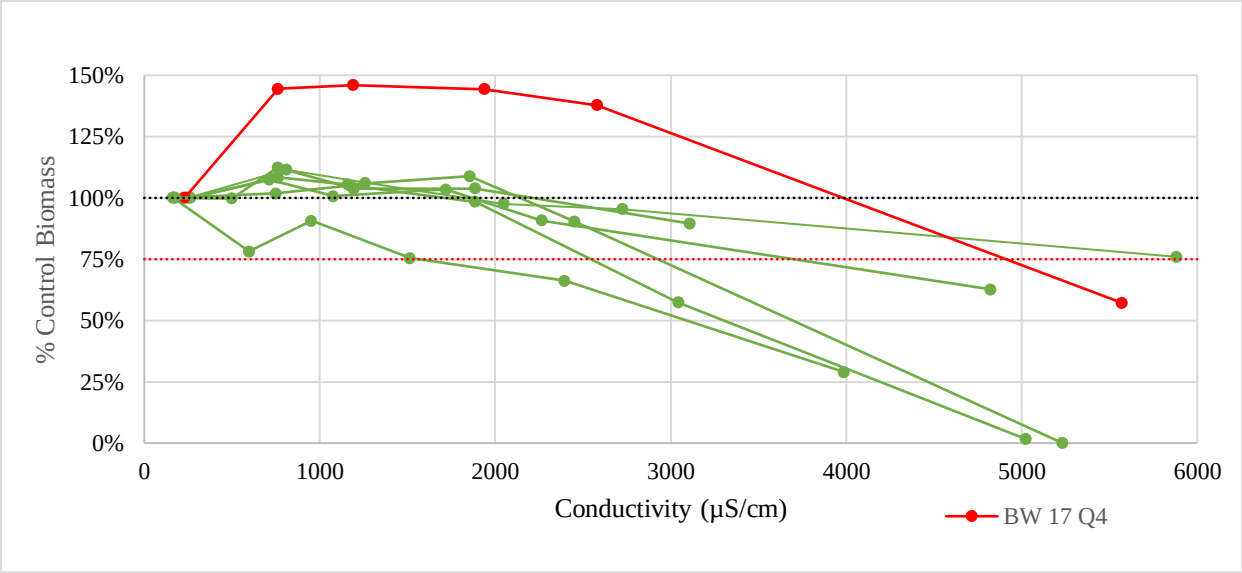

Figure 2.8 Brandywine Fathead Minnow Biomass presented as % of control growth (biomass) as determined by dry weight. Points represent treatments in dilution series (Control, 10%, 18%, 32%, 56%, 100%) as they relate to conductivity; Red line represents 2017 Q4 when lab temperature was out of compliance

The Brandywine effluent had high sulfate concentrations with fluctuating chloride concentrations. *C. dubia* are more sensitive to major ion toxicity than fathead minnows (Goodfellow et al., 2000). This is evidence that *C. dubia* reductions in reproduction from the Brandywine effluent could be major ion related. Figure 2.9 and Figure 2.10 show the relationship between conductivity and percent control reproduction for each facility's effluent. Here, conductivity was used to normalize each treatment within the three facilities. The Brandywine effluent commonly reflects a biphasic dose response curve, meaning that the 10% and 18% (and occasionally 32%) treatments outperform the control treatment. This was also evident in the Brandywine fathead minnow biomass response (Figure 2.8) and is evidence that hormesis may be occurring and low doses of the Brandywine effluent stimulate a beneficial effect. The Brandywine graph on Figure 2.9 includes a polynomial trendline that can be used to predict the conductivity at which reductions in *C. dubia* reproduction are expected to occur. There is sufficient similarity in the response to the Brandywine quarterly samples that the polynomial equation is predictive of the intersection of the trendline at which you expect to see toxicity (R^2 of 0.97). Faulkner has not demonstrated measurable toxicity enough times to create a curve and the conductivity at Westland is not a sufficient predictor of toxicity. Further testing at will increase the accuracy of this curve.

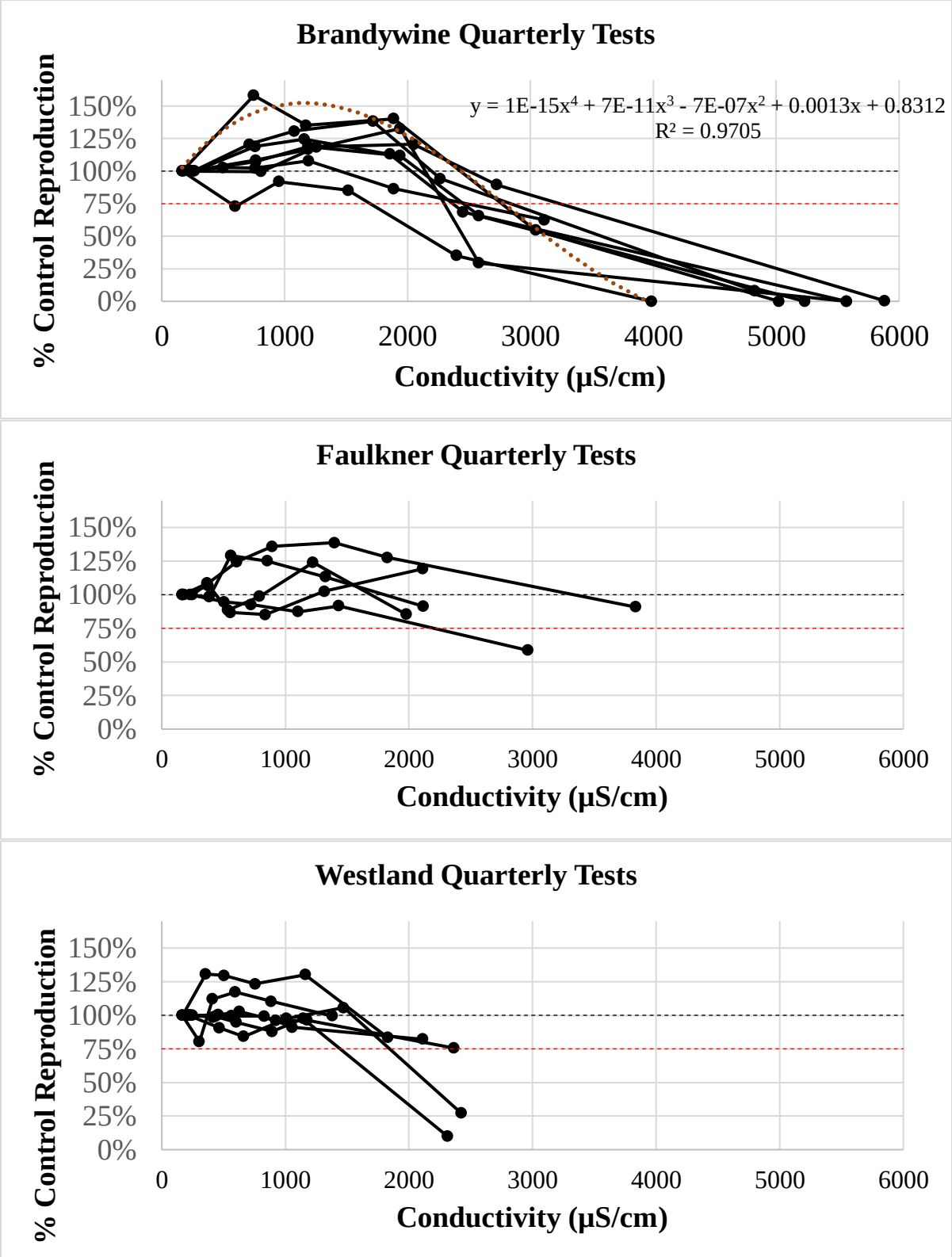

Figure 2.9 Quarterly testing of the 3 facility effluents with results presented as % of control reproduction to *C. dubia*. Points represent treatments in dilution series (Control, 10%, 18%, 32%, 56%, 100%)

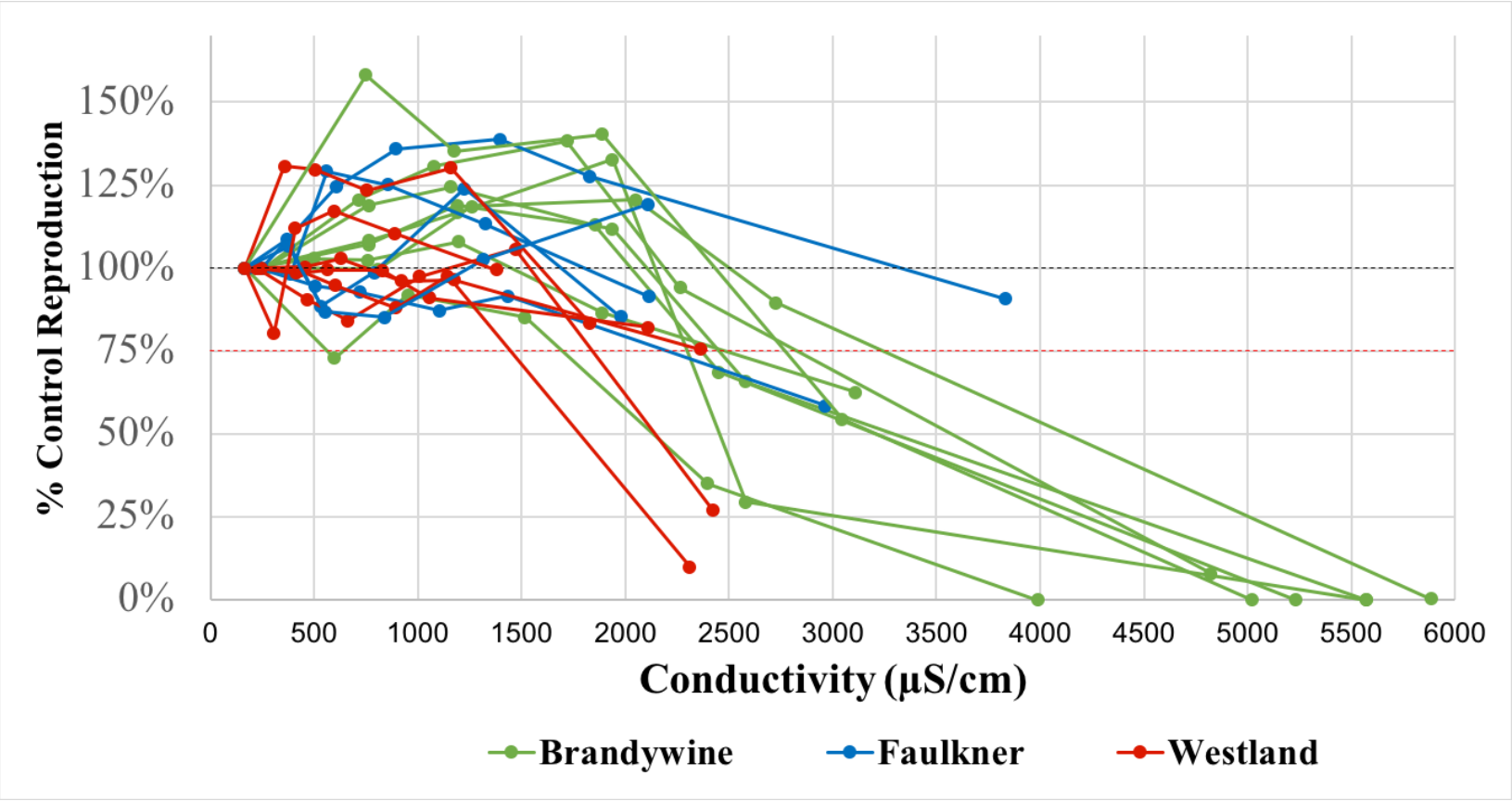

Figure 2.10 Quarterly testing of the 3 facilities *C. dubia* reproduction as a % control. Points represent treatments in dilution series (Control, 10%, 18%, 32%, 56%, 100%) and their measured conductivity

The Faulkner effluent was the least toxic of the three sites to *C.dubia* and fathead minnows. This effluent had the highest conductivity and was higher in TDS than the Westland effluents. It was less enriched with the measured major ions than Brandywine and had lower concentrations of most metals than Brandywine. Faulkner samples often showed biphasic responses in *C. dubia* reproduction in which the lower treatment concentrations outperformed the controls. The Faulkner effluent was less enriched with constituents that inhibit reproduction and growth to *C. dubia* and fathead minnows respectively than the effluents at the other two faculties. This could be a result of a more effective treatment system or the result of characteristically different leachates prior to treatment.

The Westland effluent fell in the middle of the other two sites in terms of the frequency of toxicity to *C. dubia.* The Westland effluent was unlike the other facility effluents because it was characterized by a low conductivity and low TDS relative to the other two sites. Westland samples demonstrated reductions in reproduction at conductivities that were not inhibitive of reproduction in the other samples. The biphasic dose response, suggestive of hormesis, was not seen as frequently with the Westland samples. More commonly, the 10% and 18% treatments were reduced relative to the controls (Figure 2.9 and 2.10). This suggests a different mechanism of toxicity. Something unique is happening at this site, perhaps explained by the increased selenium concentrations. Selenium toxicity is complex and dependent upon the species of selenium present. Further analysis should be done in order to characterize the toxicity occurring at Westland.

The characteristic differences at each site emphasize the need for site-specific solutions and treatment system refinements to be made. The three effluents varied in their constituents and their toxicological effects. The Brandywine effluent was characterized by high conductivity and high TDS, indicative of elevated major ions. The Faulkner effluent only reduced reproduction in *C. dubia* and reduced growth in fathead minnows at one time point, and it contained a similar makeup of major ions but at much lower concentrations and lower conductivities than the Brandywine effluent, perhaps below a toxicity threshold. The Westland treated effluent demonstrated toxicity at conductivities below which are inhibitive in the other two effluents. For this reason, the primary cause of toxicity at Westland is likely not explained by major ion toxicity alone. Commonalities can be found among the three facilities but further toxicity identification and the approach to correct toxicity will need to be unique at each facility. It cannot be determined whether or not the differences seen are due to differences in treatment systems (and their effectiveness) or differences in the leachates prior to treatment. Further analysis should be done.

Chapter 3 : Toxicity Identification Evaluation

Abstract

The treated effluent discharged at the Brandywine Coal Ash Storage facility in Prince George's County Maryland has consistently demonstrated chronic toxicity to *Ceriodaphnia dubia* through 7-day whole effluent toxicity (WET) testing. Priority metals and major ions have been measured and monitored over time and concurrently with bioassays. The discharge effluent routinely had elevated conductivity and total dissolved solids (TDS), both indicative of elevated major ions. Traditional Phase I toxicity identification evaluation (TIE) methods do not address major ion imbalances. Models for predicting the acute toxicity of a number of salt mixtures to *Ceriodaphnia dubia* have been developed, however, the thresholds at which chronic reductions in reproduction occur are still uncertain. Therefore, the present study evaluated the toxicity associated with major ion imbalances using mock effluents and a weight-of-evidence approach in order to identify the primary causative ions. The ions Ca^{2+}, Cl^{-}, Mg^{2+}, Na^{+}, K^{+}, and SO_4^{2-} were used to create a reconstituted mock effluent. A seven-day static renewal dilution series was completed using *C. dubia* and resulting threshold inhibition concentration values (IC25s) were compared between the reconstituted mock effluent and the wastewater. Subsequent toxicity tests were completed targeting single salts and salt mixtures in order to evaluate ion interactions and mechanisms of toxicity, as well as the ameliorative effects of ions in mixtures. The *Mock Synthetic Effluent* and the Brandywine discharge samples caused similar reductions in reproduction to *C. dubia*, evidence of major ions as the source of toxicity. Single ion tests were unable to confirm or eliminate any individual ion as the

primary cause of toxicity. It was, however, apparent that K toxicity is ameliorated by the ion mixture.

Introduction

The treated effluent discharged at the Brandywine Coal Ash Storage facility in Prince George's County Maryland has consistently demonstrated chronic whole effluent toxicity to *Ceriodaphnia dubia*. When toxicity is determined within an industrial effluent, regulatory agencies require a Toxicity Reduction Evaluation (TRE) in order to reduce or eliminate the sources of toxicity within an effluent. TREs prompt the start of a Toxicity Identification Evaluation (TIE), composed of 3 major objectives: to characterize the toxicant, to identify the specific toxicant or class of compounds, and to confirm that the constituent identified is the cause of the observed toxicity (G. Ankley et al., 2011; Goodfellow et al., 2000; McCulloch et al., 1993; T. Norberg-King, Dawson, & Lott, 2005; T. J. Norberg-King, Mount, Amato, & Jensen, 1992). The conventional methods employed and designed characterize toxicity due to volatile compounds, metals, oxidants, non-polar organics, filterable constituents, and ammonia. Other classes of toxicants are not well addressed by these methods; therefore, the effectiveness of these approaches varies depending on the specifics of the effluent (G. Ankley et al., 2011; Goodfellow et al., 2000; McCulloch et al., 1993; T. Norberg-King et al., 2005).

The Brandywine effluent is characterized by high total dissolved solids (TDS), high conductivity, and high concentrations of Cl, SO_4, and K, evidence of possible major ion toxicity. Toxicity caused by major ions remains difficult to address and determine, although it is one of the most prevalent causes of toxicity among

industrial effluents (Ankley et al., 2011). Initial indicators of ion imbalance as a potential source of whole effluent toxicity consider the salinity or conductivity of the wastewater, the species sensitivity to the effluent, and the failure of the USEPA Phase I TIE manipulations to reduce toxicity. Therefore, in the case of suspected major ion toxicity, previous methods applied utilized tiered approaches and the creation of synthetic effluents prepared to mimic ionic composition of the effluent (Erickson et al., 2017; Goodfellow et al., 2000; Kunz et al., 2013; McCulloch et al., 1993; Mount et al., 1997). The principal methodology of this approach is to compare toxicological responses between the synthetic effluent and the actual effluent.

Mount et al (1997) developed a multivariate regression model to predict the acute toxicity attributed to major ion imbalances on three species: *C. dubia, Daphnia magna,* and fathead minnow (*P. promelas).* The development of the model, as well as previous studies, have focused on acute toxicity. The model is helpful as a predictive tool, but it is limited in its application. The model does not consider the influence of diluent water on major ion toxicity, nor does it address complex ionic interactions or chronic sublethal endpoints. The objective of the present study was to evaluate the chronic toxicity associated with elevated major ions and the respective *C. dubia* reductions in reproduction.

Experimental Design

A *Mock Synthetic Effluent* was created to mimic discharge samples from the Brandywine Coal Ash Storage Facility in ionic composition. The purpose of the mock effluent was to evaluate if the overall toxicity of the Brandywine effluent was similar to the toxicity caused by the selected major ions alone. Similarity in the responses of

the two effluents would be evidence that major ions, or the combination of major ions, are the primary cause of the toxicity seen in the Brandywine effluent. If the toxicity of the *Mock Synthetic Effluent* was reduced relative to the Brandywine samples, then a constituent other than major ions is likely contributing to the overall toxicity.

Based on literature, Cl, SO_4, and K were predicted to be the greatest contributors to overall toxicity (Erickson et al., 2017; Goodfellow et al., 2000; Mount et al., 1997). In an attempt to identify or rule-out which constituent was the primary contributor to toxicity, three subsequent tests were completed, henceforth referred to as "*Cl^- Synthetic Effluent*," "*K^+ Synthetic Effluent*," and "*SO_4^{2-} Synthetic Effluent*" each targeting a single major ion paired with a non-suspect ion in order to investigate how single ions contribute to overall toxicity. Non-suspect ions included ions not typically thought to cause toxicity or substantially below limits at which toxicity is seen. Potassium chloride was used to match K concentrations in an attempt to identify or rule-out potassium; $NaSO_4$ was used to match SO_4 concentrations in an attempt to rule-out SO_4; and NaCl was used in an attempt to rule-out Cl. A *Mock-K^+ Synthetic Effluent* was created to mimic the *Mock Synthetic Effluent* without K. In order to keep Cl concentrations equal, extra NaCl was added to match Cl concentrations, causing the Na concentrations to increase over what was used in the *Mock Synthetic Effluent*. It was hypothesized that the toxicity to the *Mock – K^+ Synthetic Effluent* would be reduced relative to the *Mock Synthetic Effluent* because the relative ion toxicity of K is higher than that of the other ions (Mount et al., 1997).

Methods

Synthetic Effluents Preparation

The analytical results for the treated effluent from Brandywine were used to generate the target concentrations of each anion and cation in the *Mock Synthetic Effluent* (Appendix C). Salt additions were calculated based on mass balance. Reagent grade NaCl, $NaHCO_3$, Na_2SO_4, KCl, $MgSO_4$, and $CaSO_4 2H_2O$ were used to prepare each synthetic effluent, concentrations can be seen in Table 3.1 and Figure 3.1. The *Mock Synthetic Effluent* and the *K^+ Synthetic Effluent* were tested twice using the same concentrations but a slightly different dilution series (Table 3.2).

Reconstituted waters were prepared in 20-L batches by dissolving reagent-grade salts into diluted mineral water (DMW) that consisted of 20% Perrier™ and 80% distilled water. Calcium sulfate dihydrate was dissolved separately in 4 L of water and then added to 16 L of the remaining salt mixtures. Synthetic effluents were mixed with a stir bar and aerated for 48 hours prior to the start of the tests. In the cases of the *Mock Synthetic Effluent*, Ca concentrations exceeded solubility limits and did not go entirely into solution. Because of the nonvolatile nature of the test water and the small volume required each day, single batches of the dilution series were mixed at the start of the test and kept in sealed, 4-L amber bottles to be aerated and used daily for renewals. This also ensured that the concentrations of the dilution series remained consistent throughout the seven days. There was visible salt remaining at the bottom of the bottles at each concentration of *Mock Synthetic Effluent I*. Therefore, *Mock Synthetic Effluent II* and *Mock – K^+ Synthetic Effluent*

were filtered to 0.7 µm via glass fiber filtration (GFF). The Cl^-, K^+, and SO_4^{2-} *Synthetic Effluents* readily went into solution.

Table 3.1 Ionic constituents of synthetic effluent concentrations in mmol and mg/L

Mock Synthetic Effluent I and II						
Salt	**(mmol)**	**(mg/L)**	**Cation (mmol)**	**Cation (mg/L)**	**Anion (mmol)**	**Anion (mg/L)**
NaCl	24.4	1425	24.4	561	24.4	865
$NaHCO_3$	10.0	840	10.0	230	10.0	610
KCl	4.4	324	4.4	170	4.4	154
$MgSO_4$	6.2	743	6.2	150	6.2	593
$CaSO_4$ $2H_2O$	12.7	2191	12.7	510	12.7	1222
SO_4^{2-} Synthetic Effluent						
Na_2SO_4	19.7	2800	39.4	906	19.7	1894
Cl^- Synthetic Effluent						
NaCl	25.9	1515	25.9	596	25.9	919
K^+ Synthetic Effluent I and II						
KCl	4.4	324	4.4	170	4.4	154
Mock Synthetic Effluent - K^+						
NaCl	28.7	1680	28.7	660	28.7	1020
$NaHCO_3$	10.0	840	10.0	230	10.0	610
$MgSO_4$	6.2	743	6.2	150	6.2	593
$CaSO_4$ $2H_2O$	12.7	2191	12.7	510	12.7	1222

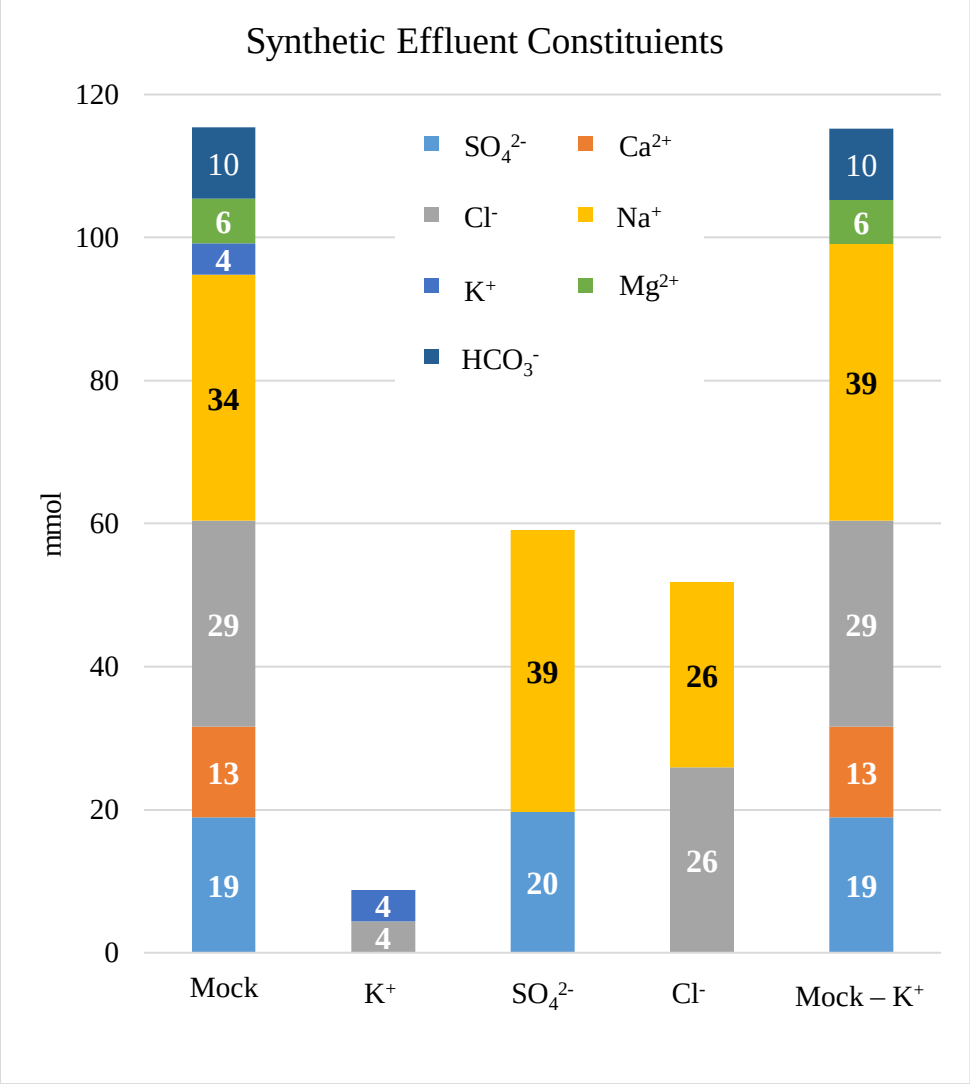

Figure 3.1 Major ion concentrations (mmol) for each synthetic effluent

Test Organisms

Neonate *C. dubia* less than 48 hours old and born within a 24-hour window of each other were cultured inhouse in DMW. Individual cultures were maintained, and neonates of known parentage were placed in treatments such that each treatment contained 10 to 12 unique individual replicates and each treatment was genetically identical to the other treatments. Parent cultures were sourced from Aquatic Biosystems Inc. in Fort Collins, CO.

Laboratory Protocols

Ceriodaphnia dubia were exposed to synthetic effluents in a seven-day static renewal system. Tests comprised of five or six treatments with a 75% or 56% dilution series (Table 3.2). Tests were maintained at 25º C in a temperature-controlled test chamber with a 16h light/8h dark light cycle. Diluted Mineral Water was used as control water and diluent.

Ceriodaphnia dubia tests were conducted using 10 to 12 test chambers at each treatment with one neonate in each chamber at the start of the test. Leached polystyrene chambers of 30 mL volumes were filled with 15 mLs of test solution. Each day, a new set of plastic chambers was prepared, and organisms were transferred from original test chambers into new chambers. Old test chambers were held overnight so that young could be counted after 24-hours had passed and young were more visible. Toxicity testing protocols followed EPA Method 1000.0 (EPA 2002). *C. dubia* were fed a 250 µL aliquot of a 1:1 mixture of algae (*Raphidocelis subcapitata)* 6.0 x 10^7 cells/ml and yeast-Cerophyll-trout chow (YCT) daily at each renewal.

The tests were completed over 2 phases (Table 3.2). Phase I occurred in April 2018 and included the *Mock Synthetic Effluent I*, the *Cl^- Synthetic Effluent*, the *K^+ Synthetic Effluent I* and the *SO_4^{2-} Synthetic Effluent*. Twelve reps were used at each treatment and a 56% dilution series was used (100%, 56%, 32%, 18%, 10%). Phase II occurred in August 2018 and included the *Mock – K^+ Synthetic Effluent*, the *K^+ Synthetic Effluent II* (10 reps at each treatment) and the *Mock Synthetic Effluent II* and a 75% dilution series (100%, 75%, 56%, 42%, 32%). Phase I indicated reductions in

reproduction at higher concentrations; therefore, the second phase was designed to focus-in on the response at higher concentration for the *Mock Synthetic Effluent (II)* by including a 75% and a 42% concentration. During the second week of Phase II 12 reps were used at each treatment and a 24% concentration was included because of an overall decrease in production and survival across all treatments. It is therefore important to consider the time separation and the differences in the dilution series and overall health of the cultures across these tests. The health of the cultures may affect the sensitivity of *C. dubia* and can lead to variation within and among laboratories (Chapman, 2000).

Table 3.2 Start dates, number of *Ceriodaphnia dubia* replicates, and dilution series employed for each synthetic effluent test

Synthetic Effluent	**Date**	**Reps/Treatment**	**Dilution Series**
Mock I	4/4/18	12	100/56/32/18/10
Cl^-	4/4/18	12	100/56/32/18/10
K^+ I	4/12/18	12	100/56/32/18/10
SO_4^{-2}	4/12/18	12	100/56/32/18/10
Mock - K^+	8/2/18	10	100/75/56/42/32
K^+ II	8/2/18	10	100/75/56/42/32
Mock II	8/9/18	12	100/75/56/42/32/24

Endpoints and Statistics

Lethality and reproduction as indicated by total neonate production served as toxicological endpoints for the *C. dubia* seven-day tests. No observable effect concentrations (NOECs) were calculated using Analysis of Variance (ANOVA). Inhibition Concentration 25s (IC25) were calculated using linear interpolation methods and used to compare toxicity between tests. All analyses were completed

using the USEPA Wet Analysis Spreadsheet v1.6.1. (https://www3.epa.gov/npdes/pubs/wet_analyticalspreadsheet.xls)

No observable effect concentrations are limited to selected treatment concentrations; therefore, the intervals are determined by the selected treatment concentrations. Independence of each test chamber was assumed. Normal data and homogeneity of variance were tested using Shapiro-Wilk test and Bartlett's test, respectively (USEPA, 2002). Tests with normally distributed data that passed homogeneity of variance with equal number of replicates were tested using Dunnett's test (USEPA, 2002). Tests with unequal number of replicates were tested using a t-test with Bonferroni adjustment. Tests with non-normally distributed data and/or heterogeneity of variance with equal number of replicates were tested using Steel's Rank Test, tests with unequal replicates were tested using Wilcoxon Rank Sum Test with Bonferroni adjustment (USEPA, 2002). Alpha levels of 0.01 were used for testing the assumptions of parametric analysis. Alpha levels of 0.05 were used to determine NOECs.

Point-estimation techniques were used to calculate IC25s. The linear interpolation method assumes responses are non-increasing, follow a linear-response function, and are from independent and representative data (USEPA, 2002). In cases in which the means of each treatment increased, these data were adjusted by smoothing. The precision of these estimates within each test was assessed by the 95% confidence intervals calculated by the bootstrap method (USEPA, 2002). Precision among tests was compared by standard deviations.

Water Quality and Chemistry Analysis

The temperature, pH, dissolved oxygen (DO), conductivity, alkalinity, hardness, and ammonia were measured in the lab at the start of each test (day 0) for both the synthetic effluent and the control water. Temperature, pH, and DO were measured for the "new" water prepared daily for each treatment, as well as the old water sampled before the renewal occurred.

Samples of each concentration of the *Mock Synthetic Effluent* and the high and low concentrations of all other synthetic effluents were taken at the start and end of each test, held in 125 mL amber bottles, and stored in the dark at 4°C for chemical analysis. Chemical analysis was completed by Microbac Laboratories Inc. in Baltimore, Maryland. Measured concentrations of major cations and anions as well as relevant water-quality parameters were compared with nominal (target) values (Appendix D).

Results and Discussion

Water Quality

Water quality parameters (pH, DO, ammonia, alkalinity) fell within normal ranges. Hardness of the *Mock Synthetic Effluent* could not be determined using ethylenediaminetetraacetic acid (EDTA) titration.

Chemistry Results

The measured concentrations of the five synthetic effluents were all within 25% of the nominal target values. Sulfate, Mg, K, and Na concentrations were within 10% of the nominal values, Cl concentrations were within 12% of the nominal values

(See appendix D). Calcium was the major ion that varied the most (25% of nominal values), due to its low solubility limits and its failure to dissolve completely in the 100% *Mock Synthetic Effluent*. The total concentrations of major ions measured at the Brandywine site suggest that this effluent is super-saturated with ionic components. This implies that rainfall within the site, as well as the dilution of these effluents through WET testing in the lab, have the potential to change the makeup of the test water and possibly affect the relationship of the major ions to each other.

The conductivity of the synthetic effluents at each treatment is given in Figure 3.2. Linear relationships in conductivities were consistent with the dilution series.

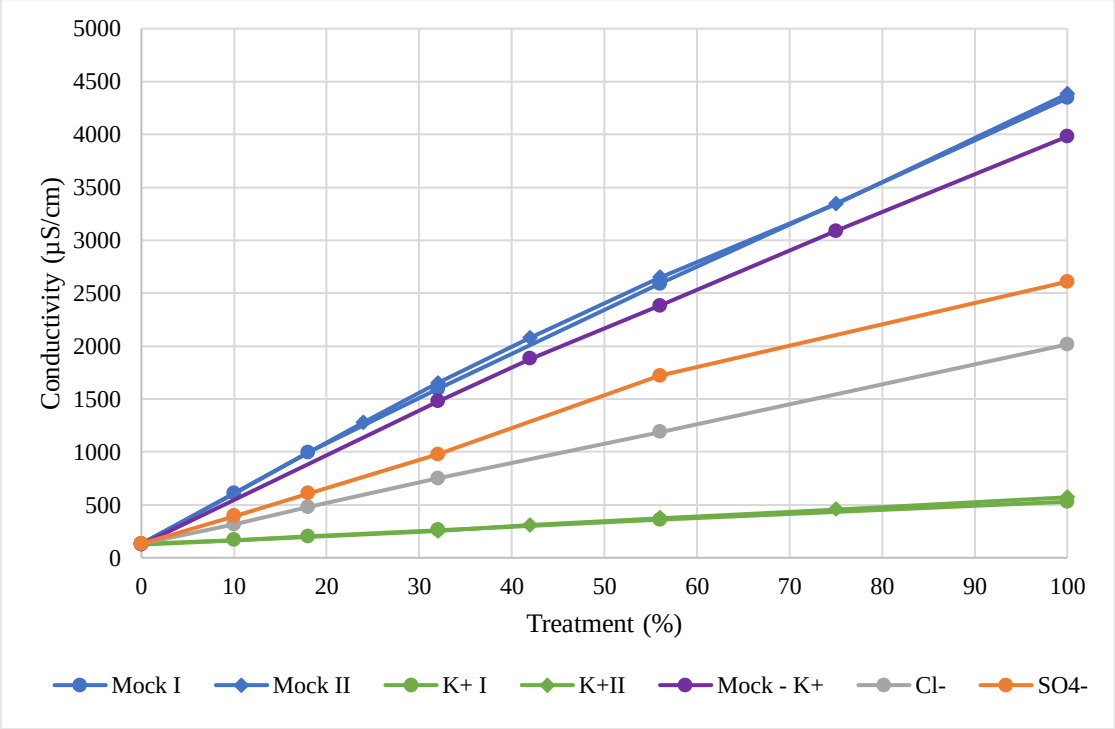

Figure 3.2 Conductivity of each synthetic effluent. Points represent each treatment concentration.

Toxicity Results

Mock Synthetic Effluent I and II

Both the *Mock Synthetic Effluent* and the *K^+ Synthetic Effluent* were generated and tested twice with a tighter dilution series used on second tests and with different *C. dubia* cultures. The *Mock Synthetic Effluent I* had a 100/56/32/18/10 dilution series and the *Mock Synthetic Effluent II* had a 100/75/56/42/32/24 dilution series. This was meant to add treatments between the higher concentrations where reductions were seen in the *Mock Synthetic Effluent I* to get a more precise measure of the range of toxicity. The IC25s based on reproduction of both of these tests were within 5% of each other (Table 3.3) The mean IC25 for the 2 tests was 48.6%. The NOEC for both trials of the *Mock Synthetic Effluent* was 56%. The 100% treatment of the *Mock Synthetic Effluent I* reached 42% mortality by day 6 (Figure 3.3). The other treatments did not experience mortality higher than 8%. The *Mock Synthetic Effluent II* demonstrated 50% mortality at the 75% treatment and 75% mortality at the 100% treatment (Figure 3.4). The higher mortality in the *Mock Synthetic Effluent II* is likely due to interlaboratory variability and the overall health of the *C. dubia* cultures at the time of the test.

Table 3.3 Chronic survival and reproduction toxicity results for each synthetic effluent test and the Brandywine samples they were modeled after

Synthetic Effluent	Date	% Survival		Reproduction							Dilution Series
		Control	NOEC	Control	NOEC	PMSD	TU	IC25	LOWER LIMIT	UPPER LIMIT	
Mock I	4/4/18	100	56	36.0	32.0	22.7	2.1	47.3	40.3	56.7	10/18/32/56/100
Mock II	8/9/18	100	56	30.5	32.0	12.4	2.0	49.9	45.5	56.2	32/42/56/75/100
	Mean	100	56	33.3	32.0	17.6	2.1	48.6	42.9	56.5	
	SD	0	0	3.9	0.0	7.3	0.1	1.8	3.7	0.3	
Mock -K^+	8/2/18	100	75	22.9	32.0	25.3	3.0	33.6	8.5	44.7	32/42/56/75/100
K^+ I	4/12/18	100	56	32.3	32.0	30.8	1.7	58.1	42.8	62.3	10/18/32/56/100
K^+ II	8/2/18	100	42	26.6	42.0	29.4	2.3	44.2	19.3	46.2	32/42/56/75/100
	MEAN	100.0	49.0	29.4	37.0	30.1	2.0	51.1	31.0	54.3	
	SD	0.0	9.9	4.0	7.1	1.0	0.4	9.8	16.6	11.4	
SO_4^{2-}	4/12/18	100.0	100.0	40.7	32.0	20.4	1.7	59.8	30.2	65.9	10/18/32/56/100
Cl^-	4/4/18	100.0	100.0	43.8	56.0	15.7	1.4	73.8	67.1	84.8	10/18/32/56/100
Brandywine	9/27/17	83.3	100	26.8	32	22.5	2.22	45.1	38.5	50.7	10/18/32/56/100
	12/13/17	100	56	28.0	32	26.8	2.58	38.7	37.8	40.4	10/18/32/56/100
	MEAN	91.7	78	24.7	32	24.7	2.40	41.9	38.15	45.6	10/18/32/56/100
	SD	11.8	31.1	0.8	0.0	3.0	0.3	4.5	0.5	7.3	10/18/32/56/100

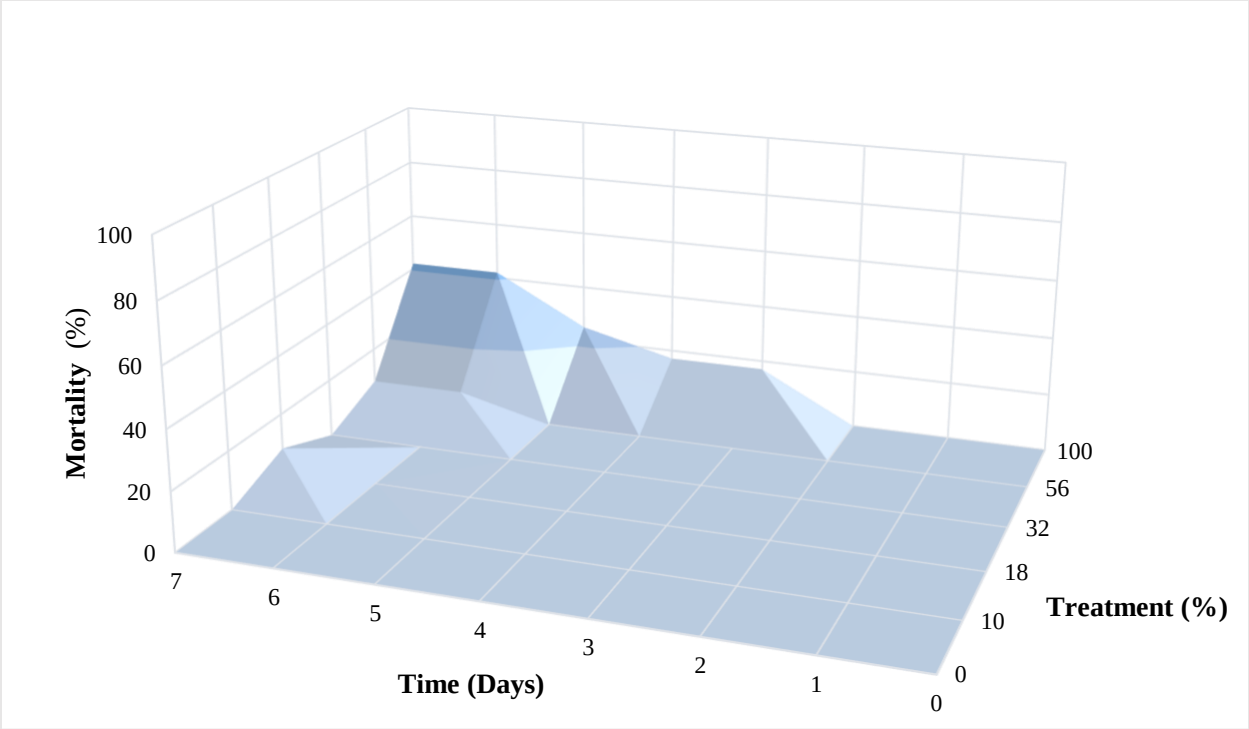

Figure 3.3 *Mock Synthetic Effluent I* percent mortality at each treatment over 7 days

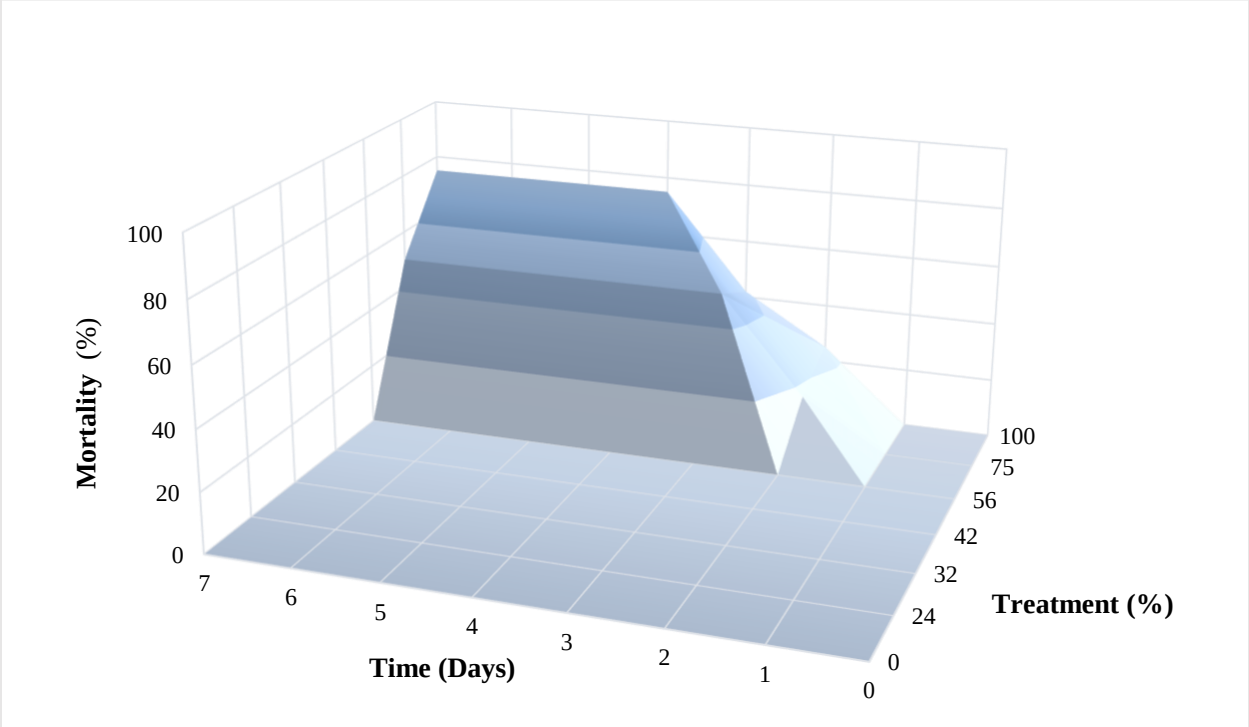

Figure 3.4 *Mock Synthetic Effluent II* percent mortality at each treatment over 7 days

Relationships Between Mock Synthetic Effluents and Brandywine

The *Mock Synthetic Effluent* was designed to mimic the Brandywine samples collected and tested in 2017 Q3-2 and 2017 Q4 for the measured concentrations of Ca, Cl, Mg, and SO_4. These quarters were selected as being the "worst case scenarios" for the data available. Potassium and Na were not measured on those dates, but concentrations were estimated based on concentrations measured in the 2018 Q1 samples. Both *Mock Synthetic Effluents* had NOECs of 56% (Table 3.4). *Mock Synthetic Effluent I* had 58% survival at the 100% concentration after 7 days and *Mock Synthetic Effluent II* had 25% survival at the 100% concentration after 7 days. This is higher mortality than seen in both Brandywine samples that had survival NOECs of 100%. The *Mock Synthetic Effluent I and II* produced similar reductions in reproduction to each other, with an average IC25 of 48.6%. The NOECs for reproduction for *Mock Synthetic Effluent I* and *Mock Synthetic Effluent II* were both 32%. The Brandywine samples had a very similar average IC25 of 41.9%. The mean IC25s for the *Mock Synthetic Effluent* and the mean of the 2017 Q3-2 and 2017 Q4 Brandywine samples were within 13.8% of one another. Figure 3.5 shows *C. dubia* reproduction for each treatment of the *Mock Synthetic Effluent* and the Brandywine effluent samples as reported as a percent of the control reproduction. Both Brandywine samples and the *Mock Synthetic Effluent I and II* show a biphasic response at the lower concentrations. The less pronounced response is seen in the *Mock Synthetic Effluent II* and is partially explained by the difference in the dilution series. The *Mock Synthetic Effluent II* dilution series consisted of 100%, 75%, 56%, 42%, 32%, and 24%, the lowest treatment was 24% and reproduction was increased

relative to the controls but not as dramatically as is seen in the 10%, 18%, and 32% of the other treatments. It was expected that the 32% treatment would be elevated compared to the controls, as it is in the other three tests. When comparing higher concentrations of the four tests, reproduction is similar. The 56% concentrations of *Mock Synthetic Effluent I and II* and both Brandywine samples were very similar. The 75% concentration was only tested in the *Mock Synthetic Effluent II*. The 100% treatment in all four tests produced no young, although the difference in survival among the tests is important. The IC25 endpoint alone does not allow comparisons between *C. dubia* that produced no young because of mortality and *C. dubia* that survived but were sufficiently impaired to not produce any young throughout the tests. Survival NOECs must also be considered in order to get an accurate picture of the response to the 100% treatments.

Figure 3.5 Reproduction of Mock I and II and Brandywine samples they were modeled after as % control reproduction. Points represent treatments in each dilution series

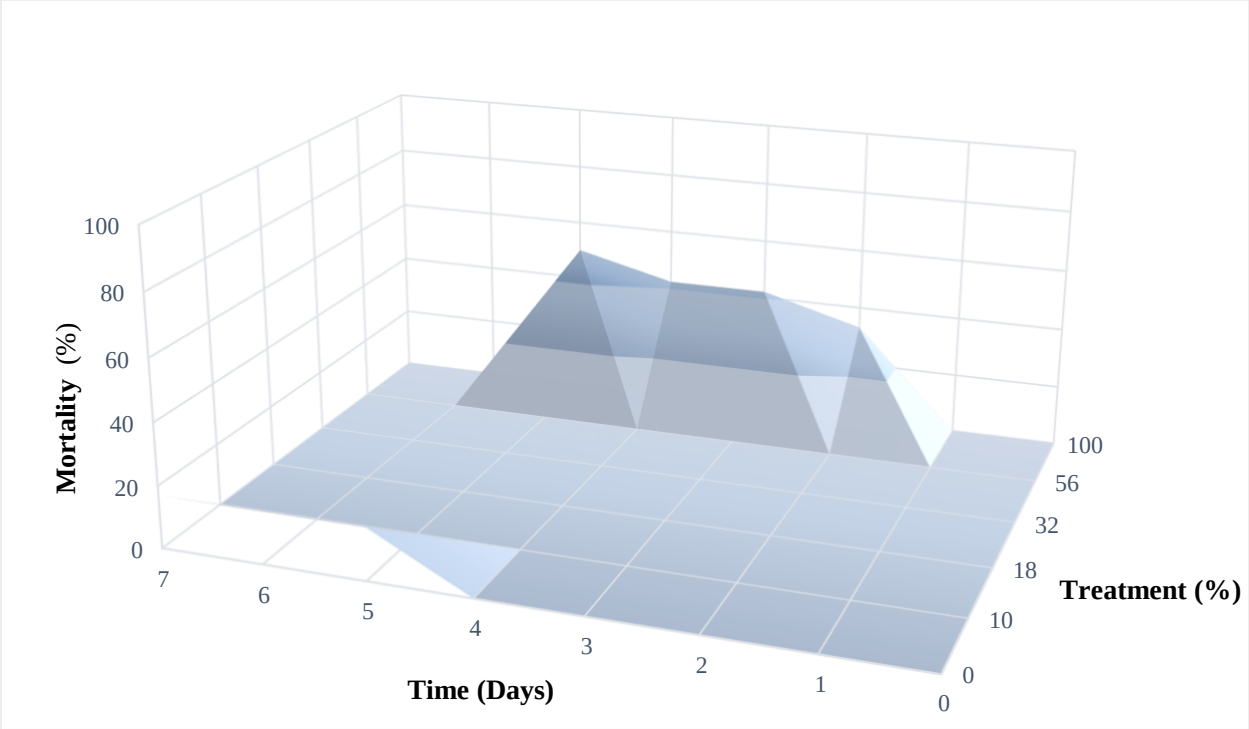

Figure 3.6 Brandywine 2017 Q3-2 percent mortality at each treatment over 7 days

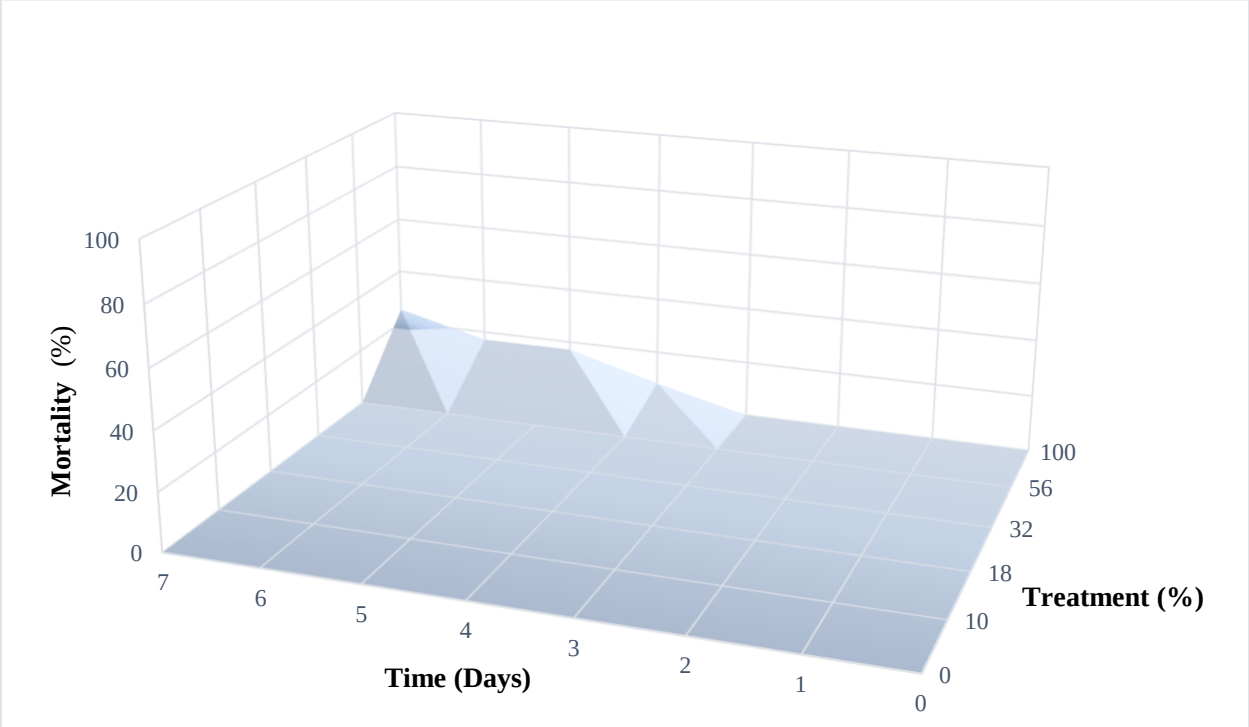

Figure 3.7 Brandywine 2017 Q4 percent mortality at each treatment over 7 days

K^+ Synthetic Effluent I and II

The two WET tests of the *K^+ Synthetic Effluent* varied more from each other than the two *Mock Synthetic Effluent* tests (Table 3.4). The *K^+ Synthetic Effluent I* completed during Phase I of the TIE utilized a 100/56/32/18/10 dilution series and the *K^+ Synthetic Effluent II* completed during phase II of the TIE utilized a 100/75/56/42/32 dilution series. The additional treatments at higher concentrations altered the IC25 and the NOEC, although the 56% treatment which was run in both dilution series varied in its response between both tests (Table 3.3). The 56% K+ treatment represents 181.44 mg/L KCl or 2.46 mmols of KCl. Complementary reference tests with KCl is used as a laboratory standard within our lab. Mortality is seen at the 250 mg/L treatment and the NOEC for mortality is 125 mg/L, the next lowest treatment. The 56%, or 181.44 mg/L, KCl treatment falls within the lab-specific threshold for survival, so the variation in response is not unusual. The age and overall health of the cultures at the start of the test could potentially affect the response at this treatment. It is also important to note that the *K^+ Synthetic Effluent I* had 12 reps per treatment and the *K^+ Synthetic Effluent II* had only 10 reps per treatment. Both of these tests were separated by time and were started with different parent cultures. The IC25 for the *K^+ Synthetic Effluent I* treatment was 58.1%, or 188 mg/L KCl and the IC25 for the *K^+ Synthetic Effluent II* treatment was 44.22%, or 143.27 mg/L KCl. The differences in the NOEC can be attributed to the different dilution series used for each test. The *K^+ Synthetic Effluent II* had a NOEC of 42% which was not a treatment used in the *K^+ Synthetic Effluent I*.

The K^+ *Synthetic Effluent* tests had an average IC25 of 51.1%, which similar to that seen in the total *Mock Synthetic Effluent*. Figure 3.8 shows the average number of young produced at each treatment of the K^+ *Synthetic Effluent* compared to the *Mock Synthetic Effluent*, plotted as a percent of the control. There is a biphasic response seen in the *Mock Synthetic Effluent I and II* as well as the K^+ *Synthetic Effluent I* at the lower treatment concentrations. The lack of a biphasic response in the K^+ *Synthetic Effluent II* can be partially explained by the different dilution series utilized. The 32% treatment used in both tests of the K^+ *Synthetic Effluent* varied in its reproductive response compared to the controls. When comparing survival, both K^+ *Synthetic Effluent* tests saw 100% mortality at the 100% treatment within 24 hours of the start of the test (Figure 3.9 and 3.10). The K^+ *Synthetic Effluent II* test saw 100% mortality in the 75% treatment within 24 hours as well, and partial mortality of the 56% treatment. The K^+ *Synthetic Effluent I* effluent had no mortality at the 56% treatment, and the 75% dilution was not a treatment. Initial results focused on reductions in reproduction to *C. dubia* as an endpoint because that was the effect most commonly seen in the Brandywine samples. When comparing the *Mock Synthetic Effluent* with the K^+ *Synthetic Effluent* tests, it became apparent that survival had to be considered as well. Both K^+ *Synthetic Effluents* were acutely lethal to *C. dubia.* The K^+ *Synthetic Effluent* is acutely more toxic than the *Mock Synthetic Effluent*, and there are likely different mechanisms of toxicity occurring. The lethality seen in the 100% *Mock Synthetic Effluent* occurred over seven-days, often later in the test when the *C. dubia* began reproducing. The lethality seen at the 100% K^+ *Synthetic Effluent* occurred acutely within 24 hours. Because the K concentration remains equal in the

Mock Synthetic Effluent and the K^+ *Synthetic Effluent*, the presence of the other constituents in the *Mock Synthetic Effluent* is mitigating K toxicity. Increased hardness and the presence of nontoxic cations within a solution have the ability to ameliorate major ion toxicity (Goodfellow et al., 2000). Sodium and to a lesser extent, Ca, are both known to ameliorate potassium toxicity (Erickson et al., 2017). This explains why the K^+ *Synthetic Effluent* saw more acute lethality than the *Mock Synthetic Effluent*.

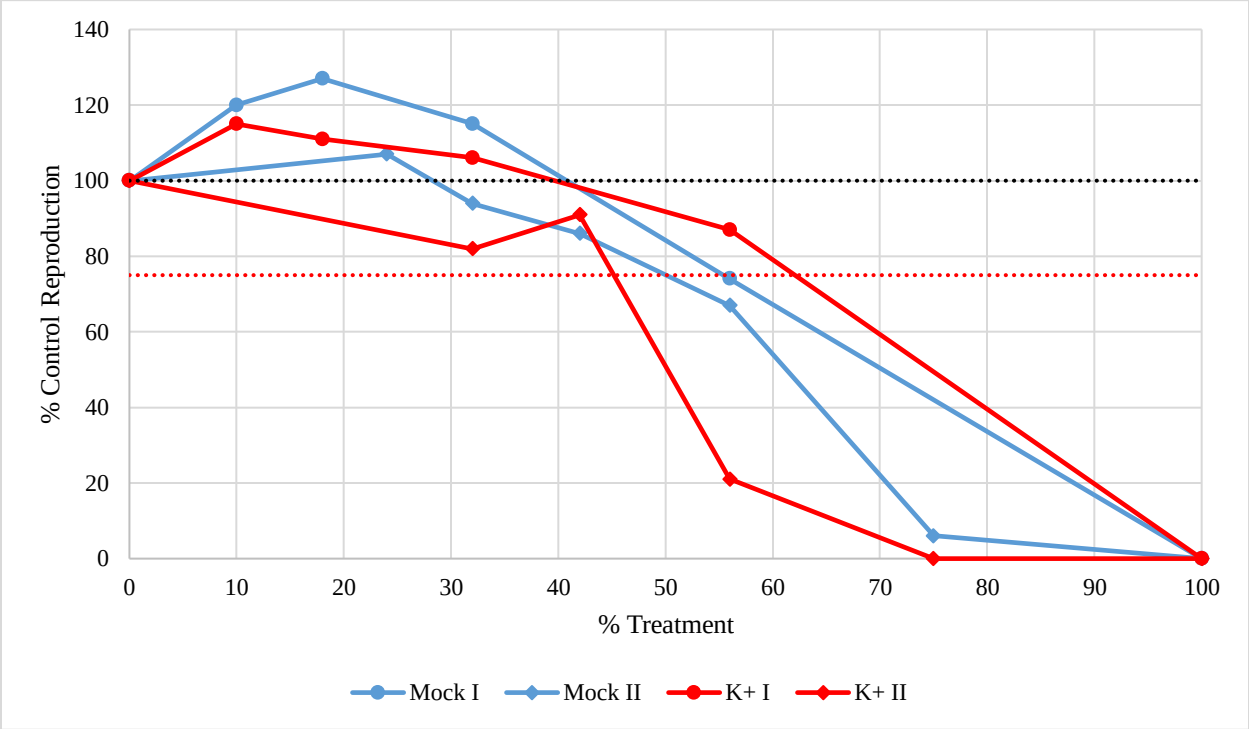

Figure 3.8 *Mock Synthetic Effluent* and K^+ *Synthetic Effluent* total reproduction of each treatment as % control reproduction. Points represent treatments in dilution series.

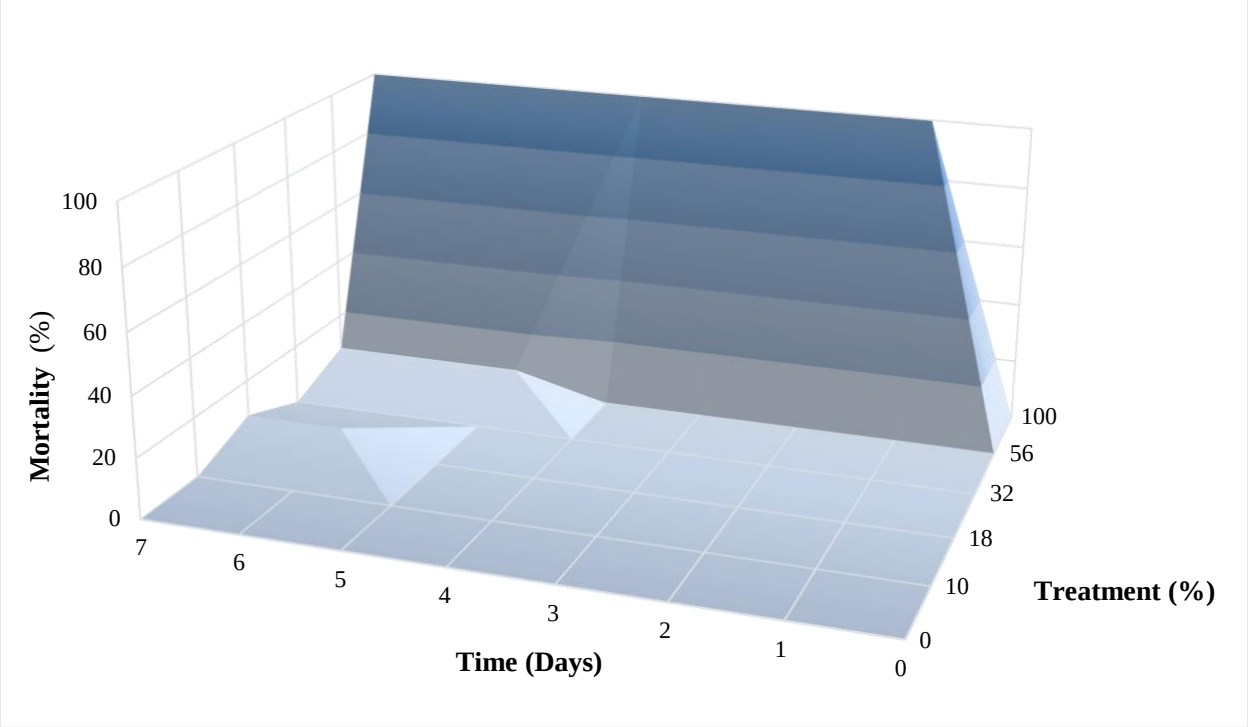

Figure 3.9 K^+ *Synthetic Effluent I* percent mortality at each treatment over 7 days

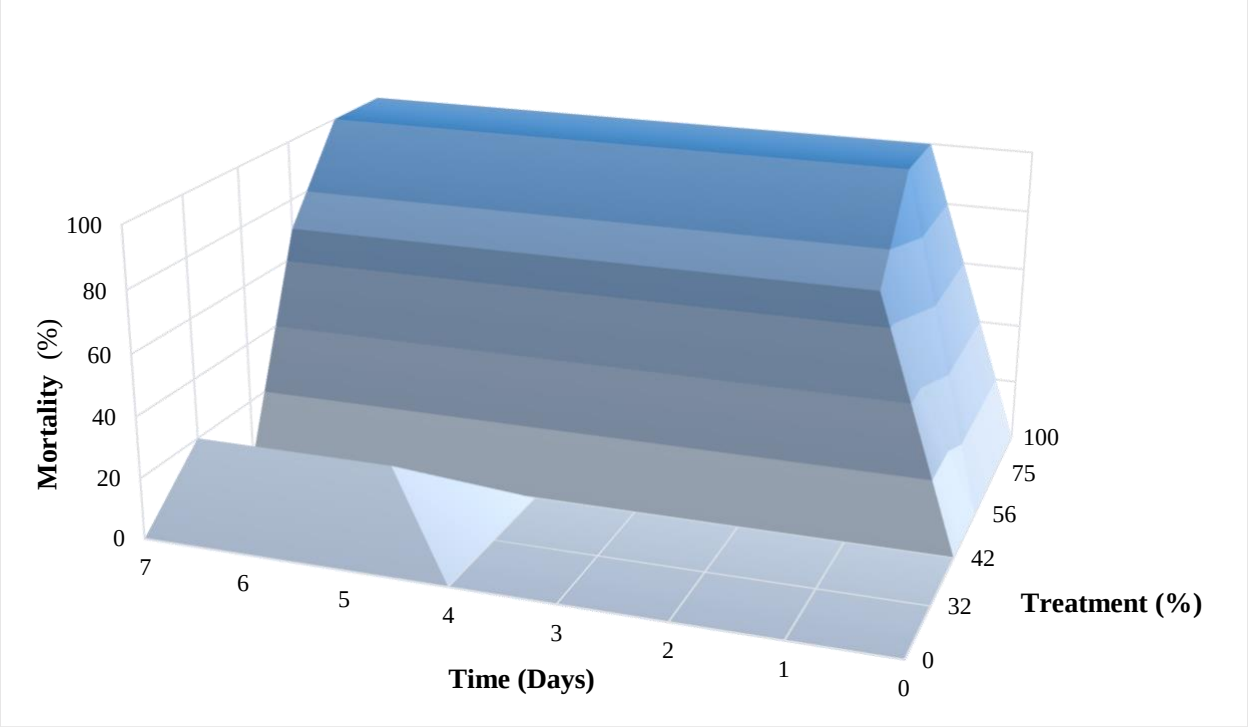

Figure 3.10 K^+ *Synthetic Effluent II* percent mortality at each treatment over 7 days

Mock-K^+ Synthetic Effluent

The *Mock – K^+ Synthetic Effluent* was created to investigate the toxicity of the remaining major ions in the absence of K. In order to keep all other salts equal, Na concentrations were increased above those that were used in the *Mock Synthetic Effluent*. Sodium is typically not considered a strong influencer on toxicity compared to the other major ions (Mount et al., 1997). The *Mock – K^+ Synthetic Effluent* was expected to be less toxic than the *Mock Synthetic Effluent*, because it was removing the K component which was hypothesized to be contributing to toxicity. The *Mock – K^+ Synthetic Effluent* had a survival NOEC of 75% and an IC25 of 33.55%. The 100% concentration had 100% mortality beginning on day 2 (Figure 3.12). The *Mock – K^+ Synthetic Effluent* caused less mortality compared to the *Mock Synthetic Effluent*, but it caused a greater reduction on reproduction compared to the *Mock Synthetic Effluent* (Figure 3.11). Further review of the literature indicated that the increase in Na from 34 mmol to 39 mmol could have been crossing a critical threshold for sodium toxicity (Erickson et al., 2017)). The *Mock – K^+ Synthetic Effluent* was not able to eliminate K as the source of toxicity to the total *Mock Synthetic Effluent*, and it determined that Na could be contributing to overall toxicity more than originally predicted.

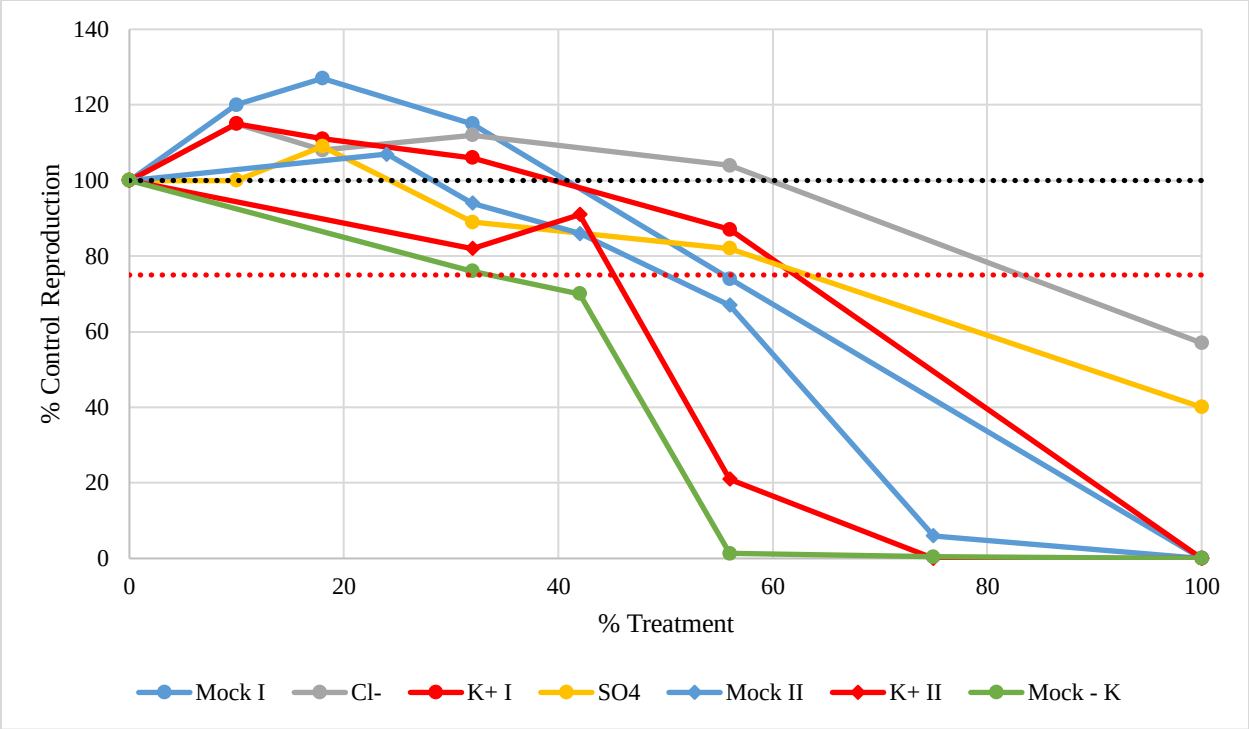

Figure 3.11 Synthetic effluent mean # of young produced as a function of percent control reproduction at each treatment of each synthetic effluent

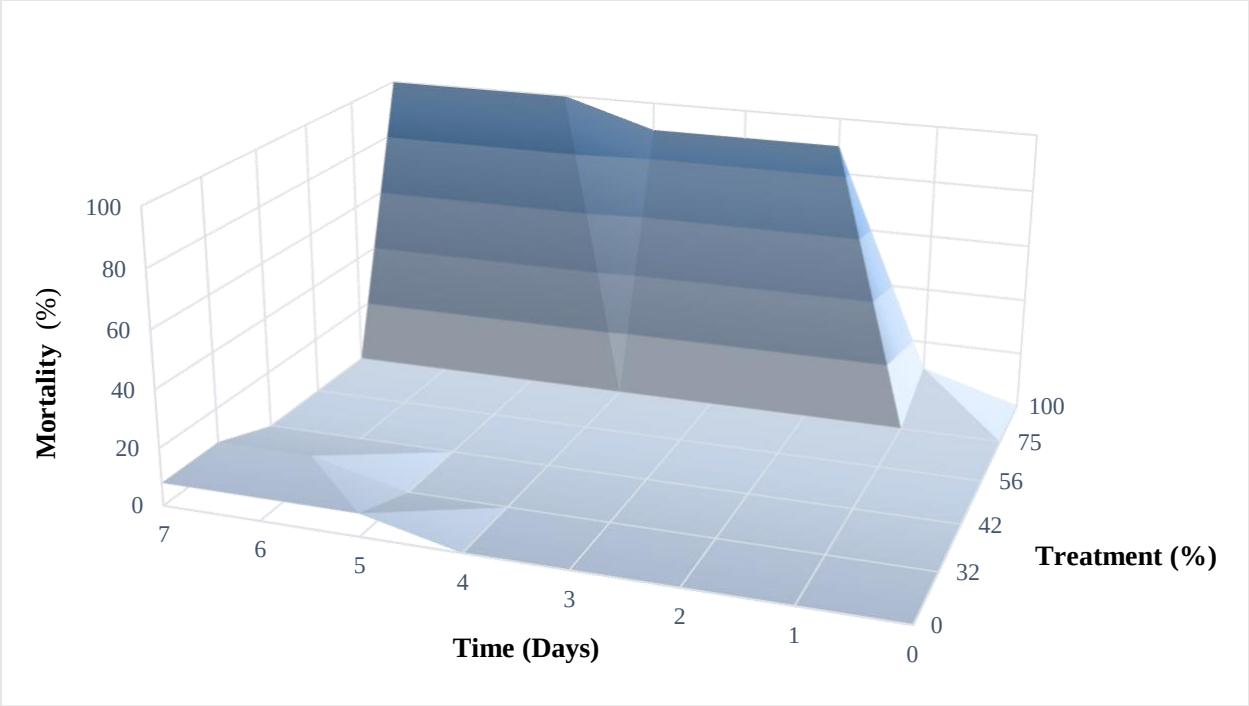

Figure 3.12 *Mock - K^+ Synthetic Effluent* percent mortality at each treatment over 7 days

SO_4^{2-} Synthetic Effluent

Sodium sulfate was used to investigate how much SO_4 contributed to the overall toxicity in the *Mock Synthetic Effluent*. The *SO_4^{-2} Synthetic Effluent* did not cause significant lethality over seven days (approximately 17%) (Figure 3.13), and therefore had increased survival relative to the *Mock Synthetic Effluent*. There was a reduction in reproduction with an IC25 of 59.76% and a NOEC of 32%. Sulfate could not be eliminated as a contributor to toxicity, but SO_4 alone is not explanatory of the toxicity seen. Sodium sulfate was selected over $CaSO_4$ and $MgSO_4$ because it is more soluble than $CaSO_4$, and Na is considered to be less toxic than Mg. (Mount et al., 1997). Further review of the literature following the tests indicated that Na could potentially be nearing a toxicity threshold. Sodium sulfate has a higher molar ratio of

Na to every mol of SO_4 than NaCl, increasing the Na concentrations to 39.4 mmol to achieve SO_4 concentrations of 19.7 mmol. It is impossible to identity if the decreases in reproduction seen (Table 3.4, Figure 3.11) are due to the increased Na concentrations or the SO_4 concentrations in the *SO_4^{-2} Synthetic Effluent*.

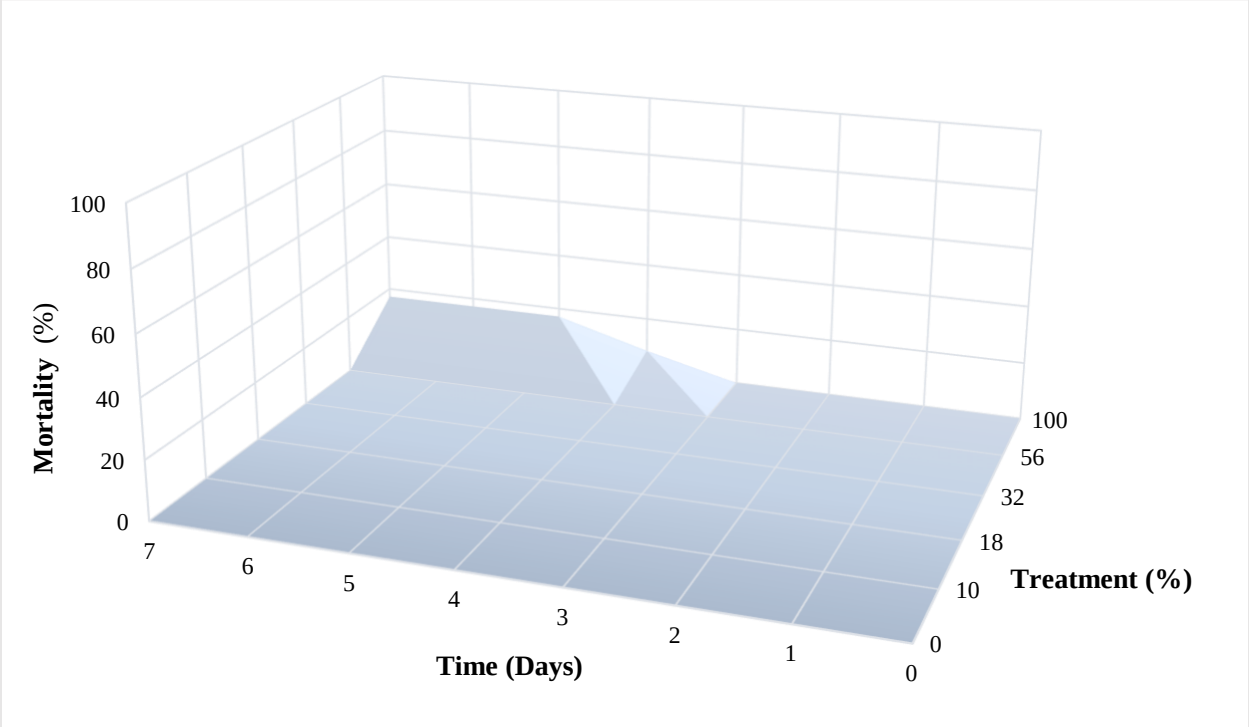

Figure 3.13 *SO_4^{-2} Synthetic Effluent* percent mortality at each treatment over 7 days

Cl- Synthetic Effluent

Like the *SO_4^{-2} Synthetic Effluent*, the *Cl^- Synthetic Effluent* did not cause seven-day mortality to *C. dubia* (Figure 3.4). The *Cl^- Synthetic Effluent* reduced reproduction with and IC25 of 73.83% and a NOEC of 56%. This is the highest IC25 of all synthetic effluents (Table 3.3). The reductions seen do not eliminate or confirm Cl as a contributor to toxicity in the *Mock Synthetic Effluent*. The relationship and

contribution of individual ions in a mixture is complex and did not allow for the elimination of a single ion as a source of toxicity.

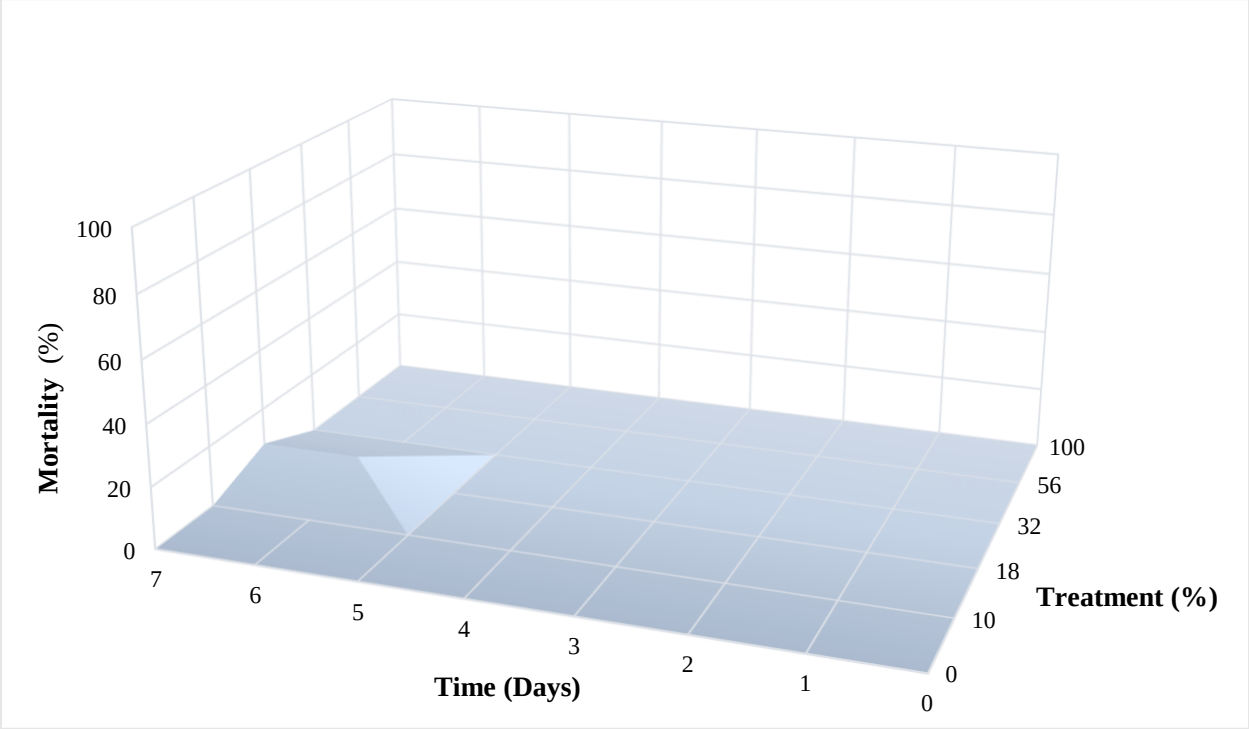

Figure 3.14 *Cl^- Synthetic Effluent* percent mortality at each treatment over 7 days

Summary and Comparison of Synthetic Effluents

The *K^+ Synthetic Effluent I and II* demonstrated acute, 24-h lethality at the 100% treatments and the 75% treatment (*K^+ Synthetic Effluent II* only) and had a varied survival response at the 56% treatment. The reductions in reproduction between the two tests were similar. The *Mock – K^+ Synthetic Effluent* demonstrated acute 48-h lethality in the 100% treatment and the greatest reductions in reproduction compared to all other synthetic effluents (IC25 33.6%) (Table 3.4, Figure 3.11). The *Mock Synthetic Effluent I and II* varied in its chronic lethality to the 100% treatment (Figures 3.3 and 3.4) but had similar reductions in reproduction. The *SO_4^{2-} Synthetic*

Effluent was less inhibitive on reproduction than the *Mock Synthetic Effluent*, but it was still found to inhibit reproduction with an IC25 of 59.8% (Table 3.4). The SO_4^{2-} *Synthetic Effluent* exhibited 17% mortality to the 100% treatment over seven days. The Cl^- *Synthetic Effluent* was the least inhibitive on reproduction (IC25 of 73.8%) (Table 3.4) and did not exhibit any mortality to the 100% treatment over seven days. The purpose of the creation of the synthetic effluents was to evaluate if the overall Brandywine toxicity was similar to the toxicity caused by major ions alone, and to determine if the major ion toxicity was comparable to the toxicity caused by K, SO_4, or Cl in order to identify or eliminate each constituent as the primary cause of toxicity. The original approach was to focus on reductions in reproduction as an endpoint. The results of Phase I and Phase II of the synthetic effluents demonstrate the need to consider all endpoints. The K^+ *Synthetic Effluent I and II* exhibited 24-hour lethality at the 100% treatment which was not seen in the other tests. The SO_4^{2-} *Synthetic Effluent* and the Cl^- *Synthetic Effluent* had complete survival at the 100% treatments and partial reproduction, and both tests of the *Mock Synthetic Effluent* had partial survival over seven days and no reproduction. These differences imply different mechanisms of toxicity occurring across the different tests and are crucial to consider. The initial design may have oversimplified the process and future tests should consider all endpoints and investigate binary salt mixtures.

Chapter 4 : Discussion

Introduction

The purpose of this project was to evaluate the treated discharge effluents of three coal ash storage facilities in Maryland. Whole Effluent Toxicity tests were completed quarterly on discharges from the Brandywine, Faulkner, and Westland ash facilities. Measurable toxicity has been observed at all three facilities, although Brandywine has consistently shown toxicity and therefore was selected to attempt nontraditional components of a Toxicity Identification Evaluation (TIE).

Brandywine

Brandywine Effluent

The Brandywine Effluent has demonstrated toxicity to *C. dubia* every quarter it has been sampled. It is characterized by high TDS, high conductivity, and high major ion concentrations. Through the creation of the *Mock Synthetic Effluent* it became apparent that the total ion concentrations exceeded solubility limits, which suggested that this effluent may be super saturated with ionic components. This suggests that increased rainfall has the potential to influence the amount and the total proportions of the ions in solution.

There was limited chemical analyses available for the 2018 calendar year, but when comparing quarterly toxicity test results over time and in relation to conductivity, it is apparent that 2018 Q2 and 2018 Q3 were the only two quarters where *C. dubia* and fathead minnows demonstrated similar IC25s. Without the

corresponding chemistry it is difficult to form a hypothesis, but this does challenge the previous hypothesis that major ion toxicity was the only contributing factor to the observed toxicity, because of the known differences in species sensitivity to major ion toxicity.

Brandywine TIE

The IC25s of the Brandywine samples and the *Mock Synthetic Effluent* were generally in very good agreement. There were limitations in the design of the *Mock Synthetic Effluent*. The effluent was designed to mimic the 2017 Q3-2 and Q4 samples, although Na and K were not analyzed on either sample (Appendix C). The 2018 Q1 samples measured Na and K but no other ions. The lowest observed conductivity and toxicity was in the 2018 Q1 samples which is evidence that perhaps the pond was different or more dilute. The *Mock Synthetic Effluent* therefore may not be a true representation of the Brandywine effluent on any given discharge.

The February sample had a sodium concentration of 730 mg/L or 32 mmol. The February sample had the lowest conductivity and toxicity and was collected after heavy rainfall. These measurements are therefore not likely representative of the overall effluent, but were used in the absence of alternative measurements. Kennedy et al. (2005) investigated the toxicity of a coal-mine effluent using synthetic effluents and attributed the toxicity to sodium and/or sulfate after comparing a Na_2SO_4 dominated effluent with an effluent formulated with Na_2SO_4, $CaSO_4$, and $MgSO_4$. Hardness was found to ameliorate Na_2SO_4 toxicity. Calcium-dominated hardness mitigates more toxicity than Mg-dominated hardness, which has been previously

documented (Jackson et al., 2000; Leblanc & Surprenant, 1984; Welsh et al., 2000). The mechanism for this mitigation has been attributed to either decreased gill permeability in the presence of Ca-rich solutions (McWilliams & Potts, 1978; Pic & Maetz, 1981) or competition at binding sites (Jackson et al., 2000). In our *Mock Synthetic Effluent*, the Na_2SO_4 concentrations were not sufficient to explain toxicity. It is possible that Na_2SO_4 toxicity could be a principle contributor to toxicity within the Brandywine effluent and that Na concentrations are much higher than we predicted. A full suite of major ion concentration data is required in order to identify dominant ions.

The subsequent toxicity tests using synthetic effluents targeting single ions were not adequate in determining or eliminating a primary causative ion. The K^+ *Synthetic Effluent* was more acutely toxic than the total *Mock Synthetic Effluent*. Sodium, and to a lesser extent, Ca, have both been known to ameliorate K toxicity (Erickson et al., 2017; Mount et al., 1997). This suggests that this type of amelioration may be occurring within the *Mock Synthetic Effluent*. Sodium toxicity was initially not thought to be of significant concern within the Brandywine Effluent. Relative ion toxicity to *C. dubia* is generally $K>>HCO_3 \approx Mg > Cl > SO_4$ (Mount et al., 1997). However, Erickson et al. (2017) determined that Na salt *C. dubia* LC50s range from 25 to 40 mmol depending on the anion. This suggests that Na could be approaching concentrations within the Brandywine effluent that are harmful; however, Ca, and to a lesser extent, Mg both have been shown to ameliorate Na toxicity (Erickson et al., 2017). Sodium is the primary cation involved in osmoregulation (Griffith, 2017). Ion transport of Na within crustaceans occurs

through an electrogenic exchanger on gill epithelia apical membranes that also transports Ca, causing elevated Ca concentrations to competitively inhibit Na uptake (Griffith, 2017). Thus, multiple mechanisms of action may be involved in major ion imbalance toxicity. Toxicity of K and Mg salts is cation-dependent and considered to be related to the chemical activities of these cations (Erickson et al., 2017). This is contrary to Na salts where both the cation and the anion contribute to toxicity. Mechanisms of Na salt toxicity are related to osmotic stress, also referred to as "nonspecific ion toxicity" (Erickson et al., 2017). Toxicity due to K is attributed to ionoregualtion and volume regulation associated with the role of K in Na transport (Griffith, 2017). The multiple mechanisms of action at play, as well as the apparent amelioration of Na and K toxicity, and the inability of our synthetic effluents to rule-out any particular ion demonstrate the complexity of ion mixtures and the need for further research.

Proposed Workplan to MDE

The pilot TIE study as well as the relevant water quality data gave evidence that the combination of major ions may be the cause of toxicity within the Brandywine effluent. The preliminary TIE was limited and not designed to be comprehensive in nature. A summary workplan to fulfill the Toxicity Reduction Evaluation (TRE) at Brandywine was submitted to MDE in August 2018 and has been approved. This is a one-year plan requiring additional chemical analysis from the facility each time the effluent pond is discharged as well as additional toxicity testing and the creation of mock effluents.

A Toxicity Reduction Evaluation is a stepwise investigation into an effluent that has demonstrated acute or chronic toxicity in order to identify the contaminant(s) responsible (Norberg-King et al., 1992). The first step of a TRE is to perform a Phase I TIE. The principle of the Phase I TIE is to manipulate the effluent to target classes of toxicants and observe how these changes influence the toxicity through chronic toxicity testing after each manipulation. Each modification therefore requires its own seven-day toxicity test. Modifications include the addition of EDTA to chelate metals; extraction through Solid Phase Extraction (SPE) columns to remove non-polar organic compounds; dechlorination with thiosulfate to remove oxidative compounds; filtration; aeration to remove volatile organic compounds; and graduated pH tests following minor pH adjustments (Norberg-King et al., 1992). Previous research suggests the EPA Phase I TIE modifications will be insufficient in reducing toxicity to the Brandywine effluent, although necessary effluent manipulations should occur (Ankley et al., 2011; Goodfellow et al., 2000; Norberg-King et al., 2005). Because the Brandywine effluent is a treated effluent unique in its makeup, several of the toxicant classes can be ruled-out. The effluent does not contain meaningful concentrations of ammonia or volatile organic compounds, and it is not treated with active chlorine or other oxidants. Previous analysis has not found non-polar organic contaminants to be present at levels of concern. Therefore, we determined the entire suite of Phase I manipulations is unnecessary.

The necessary TIE Phase I manipulations should include the addition of EDTA to chelate metals, and pH adjustment to investigate pH-dependent changes in toxicity. Chelating metals allows for investigation of possible synergistic effects and

interactions of metals with major ions. These manipulations should be completed at least twice, once in the winter and once in the summer, in the event that seasonality is affecting the characteristics of the effluent. This will indicate to what degree cationic metals contribute to observed toxicity.

All major ions must be included in the chemical analysis that occurs upon each discharge. This will allow for determination of the relationships among the major ions, and how rainfall may influence these proportions. This information, in combination with the quarterly WET tests, will be used to develop a predictive tool to estimate how often we would expect the effluent to be toxic upon discharge if no changes occur within the facility; information that is helpful regarding the nature and extent of treatment necessary to eliminate toxicity within the facility.

Quarterly WET testing will continue as before, but will now include a synthetic effluent of equal proportions of the major ions present (i.e. Ca, Cl, K, Mg, Na, SO_4) for each quarterly test. This will be generated in the same fashion as the previous *Mock Synthetic Effluent*. This will require that analytical results of the quarterly samples to be returned within one week of collection for an immediate start of the synthetic effluent tests, following completion of the regularly scheduled quarterly WET testing. The effluent characteristics (TDS, conductivity, major ion concentrations, and hardness), the measured toxicity of the Brandywine effluent and the synthetic effluent, and the species sensitivity to fathead minnow and *C.dubia* to each effluent will be used to discern the primary causative agents. Each consecutive quarter will enhance our understanding of this relationship and build on previous quarters for a comprehensive analysis of the effluent. In the instance of the mock

effluent toxicity not matching the toxicity of the Brandywine discharge, this method will be adjusted to include the addition of other metals as necessary.

Westland

Westland Toxicity

The Westland treated effluent is characterized by lower conductivities than seen at Brandywine and Faulkner, but toxicity is still apparent. Conductivity is dependent on ionic composition and is not always the best predictor of toxicity (Mount et al., 1997). The relationship and the proportion of the ions involved is critical (Erickson et al., 2017; Kunz et al., 2013; Mount et al., 2016). Sodium and K have never been measured on the Westland treated effluent. Initial steps to identify the cause of toxicity at Westland will require measuring the complete suite of major ions over time, similar to at Brandywine. The proportions are likely not the same and the differences will be critical in discerning what is contributing to toxicity, conductivity, TDS, and hardness and how they relate to each other.

Selenium is notably higher at Westland than at the other two sites, with December 2017 concentrations as high as 17.7 µg/L. Selenium is known for its narrow threshold between what is essential for life and what is toxic (Hilton et al., 1980). The literature contains a large number of studies with conflicting data on what concentrations of selenium are harmful to aquatic life. Chapman et al. (2010). determined that Se requires site-specific assessments because Se toxicity cannot be predicted in the same way as other metals and metalloids. Current research suggests that aquatic, egg laying vertebrates are the most sensitive to Se because of the large

potential for maternal transfer (Chapman et al., 2010). Risk from Se is determined by uptake at the base of the food web, dietary exposure, dietary toxicity, and trophic transfer. There is currently very poor understanding of the linkage between dissolved Se concentrations and toxicity (Stewart et al., 2010). Because Se is an essential element, cells have evolved specific high-affinity uptake pathways. Algae (green algae, cyanobacteria, diatoms) accumulate both organic and inorganic Se at low ambient concentrations (Stewart et al., 2010). Algae can bioconcentrate Se 10^6-fold higher than concentrations present in ambient water (Baines & Fisher, 2001). Bacteria have also been shown to accumulate selenite (SeIV) from water (S. Baines et al., 2004; Foda, Vandermeulen, & Wrench, 1983; Riedel, Sanders, & Gilmore, 1996). It is possible that the algae *Raphidocelis subcapitata* and yeast-Cerophyll-trout chow (YCT) within the test chambers exposed to the Westland treated effluent are accumulating Se in concentrations that are inhibiting reproduction to *C. dubia.*

Because of the possibility that Se is contributing to toxicity at Westland, a TIE investigating major ions should include Se. Application of EDTA is the TIE Phase I manipulation intended to chelate metals. However, while EDTA has been effective at reducing toxicity of Cu, Cd, Ph, Mn, Ni, and Zn to *C. dubia*, it has not been shown to effectively reduce the toxicity of Ag, Se (sodium selenite or sodium selenite), Al, or As to *C. dubia* when tested in moderately hard water (Hockett & Mount, 1996; T. J. Norberg-King et al., 1992). Selenium is an anion and is therefore not expected to complex with EDTA which is also anionic (Hockett & Mount, 1996). A Westland TIE should therefore include the synthetic effluent approach recommended for Brandywine. One possibility would be to create a mock effluent with all of the major

ions in the correct proportions without Se and compare the results to a second mock effluent that matches major ions with the addition of Se. Another possibility would be to create a single major ion mock effluent dilution series and to keep Se concentrations constant across all treatments, so that the major ions would be reduced across treatments and Se concentrations would remain the same. This would give insight into the overall contribution of selenium and the major ions to toxicity.

The observed differences in toxicity between Brandywine and Westland point to the need for site-specific TIEs and site-specific toxicity reduction plans. The occasional toxicity at Faulkner demonstrates the need for continued monitoring of these sites.

Implications and Recommendations for Mitigating Toxicity

Few solutions are in place for mitigating toxicity due to major ion imbalances. Reverse osmosis (RO) is commonly used in water treatment and desalinization. It involves a process in which water passes through a membrane from an area of high concentration of solutes to an area of low concentration of solutes (Shannon et al., 2009). This process can be energy-intensive and expensive (Shannon et al., 2009). Ion-exchange treatment methods may also be employed. Ion exchange occurs when an ion pair exchanges anions or cations with another ion pair. It is often used in water softening treatment to remove Ca and Mg ions. The treatment systems are made up of cartridges containing beads of porous plastic resin that binding ions (commonly carboxylate) are chemically bound to (Giblert et al., 2009). Carboxylate is anionic, so it will bind Mg and Ca. Hard water softened with ion exchange treatment systems

often results in soft water enriched with Na (Giblert et al., 2009). These treatment systems are expensive to maintain, and the resin creates waste products that can be difficult to dispose. Distillation is another option to reduce major ions within a wastewater. Like RO and ion exchange, distillation is expensive and energy-intensive. Some of these treatments may already be employed within the leachate treatment system, we do not have information on the specifics of the active and passive treatment that occurs at each facility.

Total dissolved solids toxicity is not necessarily a major environmental concern. Major ions are non-bioaccumulative and do not pose a major risk to human health (Goodfellow et al., 2000). In addition, these effluents may be rapidly diluted to concentrations within acceptable limits upon discharge to nearby streams (Dorn & van Compernolle, 1995). Site-specific assessments are required to evaluate the receiving water. Physical and biogeochemical properties will determine the risk to aquatic ecosystems. At some sites, it may be reasonable to consider the mixing zone and how far downstream elements are conserved (Barbour et al., 1995; Dorn & van Compernolle, 1995; USEPA, 1994). Ultimately, the decisions will be site-specific and require continued monitoring to evaluate the risk to the surrounding ecosystem.

Appendices

Appendix A: Sample Water Quality Table from Brandywine Quarterly Discharge
Appendix B: Quarterly Discharge Reproduction Raw Data
Appendix C: Brandywine Major Ion Available Analytes and *Mock Synthetic Effluent* Targets
Appendix D: Measured Concentrations of TIE Synthetic Effluents
Appendix E: TIE Synthetic Effluent Reproduction Raw Data

Appendix A: Sample Water Quality of Brandywine Quarterly Discharge WET (Brandywine 2018 Q2)

Fathead Minnow Water Quality Bench Sheet (EPA METHOD 1002.0)

	TRTMNT	Day 0	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	SUMMARY DATA			
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	MEAN	S.D.	MIN.	Max.
pH (S.U.)	C	8.04	8.36	8.26	7.97	8.14	7.03	8.22	8.03	8.14	8.00	8.14	7.48	7.91	8.17			7.03	8.36
	10%	7.98	8.19	8.18	7.83	8.07	7.59	8.19	7.82	8.09	7.97	8.00	7.32	7.85	7.92			7.32	8.19
	18%	8.04	8.10	8.21	7.83	8.08	7.72	8.14	7.82	8.08	7.95	7.97	7.38	7.83	7.84			7.38	8.21
	32%	8.08	8.10	8.22	7.84	8.09	7.69	8.12	7.79	8.10	7.90	8.03	7.33	8.18	7.97			7.33	8.22
	56%	8.11	8.11	8.23	7.98	8.13	7.79	8.09	7.85	8.11	7.93	8.07	7.58	8.31	7.88			7.58	8.31
	100%	8.14	8.16	8.25	8.04	8.09	8.07	8.05	7.97	8.10	7.93	8.12	8.01	8.26	8.32			7.93	8.32
Temp. (°C)	C	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25		25	25
	10%	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25		25	25
	18%	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25		25	25
	32%	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25		25	25
	56%	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25		25	25
	100%	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25		25	25
Diss. Oxygen (mg/l)	C	7.3	7.0	7.8	6.0	7.2	5.4	6.3	6.5	8.0	6.2	7.7	7.2	8.4	6.6	7.0	0.8	5.4	8.4
	10%	7.3	7.0	7.8	5.1	6.8	5.2	6.4	6.6	8.0	6.4	7.7	5.7	7.0	5.9	6.6	0.9	5.1	8.0
	18%	7.2	6.9	7.6	5.4	6.7	5.2	6.5	6.4	7.8	6.1	7.8	6.4	7.6	5.9	6.7	0.9	5.2	7.8
	32%	7.2	7.0	7.4	5.5	7.2	5.6	6.4	6.0	7.9	6.1	7.8	5.4	6.6	6.2	6.6	0.8	5.4	7.9
	56%	7.1	6.9	7.4	5.9	6.9	6.4	7.8	6.1	8.0	6.3	7.8	5.0	6.6	6.6	6.8	0.8	5.0	8.0
	100%	7.2	6.7	7.4	5.4	6.6	5.5	7.5	6.2	8.0	6.0	7.9	6.6	6.2	7.5	6.8	0.8	5.4	8.0
Cond. (uS/cm)	C	125	132		125		122		128		130		130			127	3.6	122	132
	10%	461	467		445		456		469		460		462			460	7.9	445	469
	18%	735	734		732		735		730		733		730			733	2.1	730	735
	32%	1158	1170		1171		1167		1164		1172		1160			1166	5.5	1158	1172
	56%	1852	1732		1858		1870		1860		1858		1875			1844	49.8	1732	1875
	100%	3062	3096		3052		3048		3071		3075		3063			3067	16.1	3048	3096
Replicate Measured		S	A	S	B	S	C	S	D	S	A	S	B	S	C				
Initials		SE	SE		SE		SE		SE		SE		SE		SE				
Changes & Notes																			

Ceriodaphnia Dubia Water Quality Bench Sheet

		Day 0	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	SUMMARY DATA			
TRTMNT		Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	MEAN	S.D.	MIN.	Max.
pH (S.U.)	C	8.04	8.39	8.26	7.78	8.14	8.18	8.22	8.25	8.14	7.81	8.14	7.87	7.91	7.92			7.78	8.39
	10%	7.98	8.34	8.18	7.86	8.07	8.16	8.19	8.20	8.09	8.11	8.00	7.95	7.85	7.88			7.85	8.34
	18%	8.04	8.35	8.21	8.14	8.08	8.13	8.14	8.28	8.08	8.10	7.97	8.00	7.83	7.94			7.83	8.35
	32%	8.08	8.32	8.22	8.12	8.09	8.17	8.12	8.15	8.10	8.16	8.03	8.12	8.18	7.96			7.96	8.32
	56%	8.11	8.30	8.23	8.16	8.13	8.24	8.09	8.15	8.11	8.21	8.07	8.05	8.31	7.90			7.90	8.31
	100%	8.14	8.32	8.25	8.23	8.09	8.29	8.05	8.21	8.10	8.12	8.12	8.14	8.26	7.89			7.89	8.32
Temp. (°C)	C	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25		25	25
	10%	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25		25	25
	18%	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25		25	25
	32%	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25		25	25
	56%	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25		25	25
	100%	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25		25	25
Diss. Oxygen (mg/l)	C	7.3	7.2	7.8	7.2	7.2	7.9	6.3	7.9	8.0	7.7	7.7	7.8	8.4	7.7	7.6	0.5	6.3	8.4
	10%	7.3	7.6	7.8	7.2	6.8	8.3	6.4	7.9	8.0	8.4	7.7	7.8	7.0	7.8	7.6	0.6	6.4	8.4
	18%	7.2	7.7	7.6	6.9	6.7	8.4	6.5	8.2	7.8	8.6	7.8	8.3	7.6	7.8	7.7	0.6	6.5	8.6
	32%	7.2	7.6	7.4	7.6	7.2	8.0	6.4	8.5	7.9	7.6	7.8	8.7	6.6	7.6	7.6	0.6	6.4	8.7
	56%	7.1	7.8	7.4	7.5	6.9	7.8	7.8	8.3	8.0	7.7	7.8	8.4	6.6	7.7	7.6	0.5	6.6	8.4
	100%	7.2	7.5	7.4	6.9	6.6	7.9	7.5	8.1	8.0	8.1	7.9	8.3	6.2	7.8	7.5	0.6	6.2	8.3
SN	C	125		132		125		122		128		130		130		127	3.6	122	132
	10%	461		467		445		456		469		460		462		460	7.9	445	469
	18%	735		734		732		735		730		733		730		733	2.1	730	735
	32%	1158		1170		1171		1167		1164		1172		1160		1166	5.5	1158	1172
	56%	1852		1732		1858		1870		1860		1858		1875		1844	49.8	1732	1875
	100%	3062		3096		3052		3048		3071		3075		3063		3067	16.1	3048	3096
Replicate Measured		S	1	S	2	S	1	S	2	S	1	S	2	S	1	NOTE: FINAL D.O. VALUES >8.3 mg/L (saturation) may occur due to photosynthetic activity of alagal food			
Initials		SE	SE		SE		SE		SE		SE		SE		SE				
Changes & Notes																			

TRTMNT		Day 0	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	SUMMARY DATA			
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	MEAN	S.D.	MIN.	Max.
Aklanity	C	75		75		75		75		75		75		75		75	0	75	75
	100%	157.5		157.5		157.5		157.5		157.5		157.5		155		157.143	0.9449	155	157.5
Hard	C	90		90		90		90		90		90		90		90	90	90	90
	100%	no endpoint reached																	
NH3	C	0.00	0.72																
	100%	0.05	0.65																

Appendix B: Quarterly Discharge Reproduction Raw Data

Brandywine 2017 Q2

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	4	34	16	21	23	0
2	30	28	29	30	0	1
3	27	30	31	30	27	0
4	24	33	23	21	10	1
5	30	35	22	31	30	5
6	32	21	27	24	25	0
7	0	35	12	33	28	0
8	0	32	39	27	15	0
9	34	0	37	29	0	4
10	0	0	36	29	28	4
11	30	7	22	24	28	0
12	27	32	17	30	10	4
Mean	19.8333333	23.9166667	25.9166667	27.4166667	18.6666667	1.58333333
Std	14.1731714	13.6612147	8.75378706	3.98767039	11.1952371	2.02072594

Brandywine 2017 Q3-1

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	34	25	28	21	13	0
2	29	22	27	30	28	0
3	27	22	27	40	15	0
4	20	22	22	20	21	0
5	21	29	34	33	19	1
6	29	26	28	33	16	0
7	20	5	21	22	22	0
8	20	27	23	18	18	0
9	22	31	22	26	31	0
10	17	25	32	24	31	0
11	19	24	29	33	11	0
12	18	17	34	33	22	0
Mean	23	22.9166667	27.25	27.75	20.5833333	0.08333333
Std	5.37671748	6.72117865	4.5751304	6.85068013	6.65320611	0.28867513

Brandywine 2017 Q3-2

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	8	33	25	32	17	0
2	27	22	34	28	30	0
3	31	30	39	36	17	0
4	34	30	41	21	15	0
5	18	29	24	23	14	0
6	34	28	38	37	26	0
7	33	37	44	38	22	0
8	19	40	25	28	14	0
9	39	32	30	25	21	0
10	21	33	39	33	16	0
11	25	32	37	39	15	0
12	33	37	25	24	14	0
Mean	26.8333333	31.9166667	33.4166667	30.3333333	18.4166667	0
Std	8.92222285	4.75697255	7.25457014	6.32934484	5.2476546	0

Brandywine 2017 Q4

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	0	0	0	0	0	0
2	0	27	30	25	0	0
3	0	0	0	0	0	0
4	38	33	33	38	25	0
5	0	0	0	0	0	0
6	27	26	23	24	19	0
7	28	29	31	25	23	0
8	0	0	0	0	0	0
9	26	21	29	23	16	0
10	22	23	30	26	16	0
11	28	24	27	34	12	0
12	27	27	30	24	18	0
Mean	16	18	19	18	11	0
Std	14.8589326	13.2630725	14.53809	14.1750421	10.0374299	0

Brandywine 2017 Q4
Retest

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	30	22	19	36	0	0
2	36	44	41	41	0	0
3	40	22	45	42	6	0
4	15	40	39	37	8	0
5	38	40	44	36	6	0
6	26	46	45	37	17	0
7	22	24	31	33	18	0
8	27	22	20	47	12	0
9	31	29	40	35	5	0
10	22	30	27	30	8	0
11	24	29	26	37	19	0
12	25	16	15	35	0	0
Mean	28	30	33	37	8	0
Std	7.33608764	9.89337091	11.0151411	4.42787315	6.9167123	0

Brandywine 2018 Q1

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	27	29	29	30	27	22
2	30	32	31	32	23	18
3	35	36	32	37	29	19
4	30	30	36	40	33	17
5	39	38	38	39	29	19
6	37	38	34	36	30	22
7	33	34	33	36	15	24
8	33	32	35	35	31	18
9	36	33	30	33	25	18
10	34	35	30	36	32	22
11	35	33	34	36	33	21
12	24	34	40	34	33	26
Mean	32.75	33.6666667	33.5	35.3333333	28.3333333	20.5
Std	4.30908132	2.80691786	3.37099931	2.80691786	5.29722626	2.77979725

Brandywine 2018 Q2

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	42	24	20	27	12	0
2	42	37	43	38	24	0
3	15	30	38	27	6	0
4	36	13	21	42	9	0
5	22	23	22	21	13	0
6	25	18	17	8	10	0
7	41	19	22	24	3	0
8	36	35	43	37	16	0
9	36	21	42	38	14	0
10	36	21	36	20	9	0
Mean	33.1	24.1	30.4	28.2	11.6	0
Std	9.25502866	7.65143414	10.8443534	10.5809052	5.7965507	0

Brandywine 2018 Q3

Rep	Cont	0.1	0.18	0.32	0.56	1
1	13	43	39	37	6	0
2	16	38	35	38	8	0
3	31	34	38	35	17	0
5	26	42	41	39	20	0
6	33	44	38	39	19	0
7	29	42	36	40	13	0
8	25	33	17	32	9	0
9	28	38	24	27	20	0
10	26	40	38	34	10	0
11	26	46	36	34	16	0
MEAN	25.3	40	34.2	35.5	13.8	0
SD	6.25477595	4.24264069	7.59824541	3.97911213	5.28730135	0

Faulkner 2017 Q2

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	15	26	26	30	0	0
2	24	0	35	26	23	22
3	0	27	29	26	26	24
4	3	29	28	25	28	19
5	10	37	31	24	0	22
6	18	30	28	0	24	34
7	22	25	32	29	23	16
8	0	34	26	23	29	23
9	31	23	28	33	23	20
10	22	33	31	27	22	29
11	25	29	15	24	6	25
12	21	26	24	23	28	21
Mean	15.9166667	26.5833333	27.75	24.1666667	19.3333333	21.25
Std	10.3963134	9.29768626	5.01135075	8.18905404	10.7984286	8.17006732

Faulkner 2017 Q3

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	12	22	22	27	31	11
2	19	23	27	19	17	18
3	16	25	23	27	29	22
4	20	25	25	28	20	16
5	19	22	22	26	22	17
6	17	24	28	26	24	9
7	18	23	24	23	25	13
8	19	26	27	17	22	24
9	26	19	21	24	19	12
10	11	18	24	31	21	19
11	19	23	23	27	27	18
12	21	20	29	26	20	18
Mean	18.0833333	22.5	24.5833333	25.0833333	23.0833333	16.4166667
Std	3.94181161	2.46797672	2.60971379	3.8720052	4.23101822	4.46111143

Faulkner 2017 Q4

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	36	36	36	23	33	17
2	42	40	28	40	35	19
3	45	38	37	26	37	21
4	37	38	39	38	40	23
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	41	39	38	40	35	12
8	32	22	31	35	32	18
9	20	35	27	24	21	26
10	40	42	37	34	38	26
11	39	30	35	32	34	26
12	37	29	34	30	33	28
Mean	30.75	29.0833333	28.5	26.8333333	28.1666667	18
Std	15.6735156	14.6750148	13.8399947	13.7829495	13.9533856	9.59166305

Faulkner 2018 Q1

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	31	30	38	39	30	25
2	34	32	37	34	39	35
3	26	0	39	32	31	27
4	35	36	34	28	30	29
5	0	34	36	34	34	30
6	31	38	41	34	37	16
7	27	3	32	37	30	25
8	35	34	43	45	28	27
9	27	33	34	36	25	25
10	34	36	36	35	33	30
11	45	36	44	41	39	24
12	22	29	34	39	37	24
Mean	28.9166667	28.4166667	37.3333333	36.1666667	32.75	26.4166667
Std	10.8414888	12.8520132	3.79792607	4.44835686	4.51512609	4.60154783

Faulkner 2018 Q2

Treatment						
Rep	Cont	10%	18%	32%	56%	100%
1	45	36	30	42	37	31
2	41	38	9	23	41	20
3	29	28	32	23	22	17
4	33	22	29	32	29	17
5	38	33	46	25	43	24
6	30	24	25	32	36	27
7	22	42	24	50	40	34
8	2	36	39	35	52	26
9	34	38	20	28	38	41
10	39	43	23	19	50	30
Mean	31.3	34	27.7	30.9	38.8	26.7
Std	12.2388271	7.19567771	10.2203501	9.52715417	8.90443086	7.66014215

Faulkner 2018 Q3

Rep	Cont	10%	18%	32%	56%	100%
1	28	36	29	18	37	38
2	40	36	38	27	28	38
3	37	32	35	34	8	14
4	27	23	27	28	22	35
5	18	22	21	17	14	23
6	22	33	25	37	38	37
7	28	35	36	31	42	35
8	27	28	23	23	24	39
10	33	38	22	19	43	37
11	23	27	20	20	36	39
12	35	29	0	17	34	44
MEAN	28.9090909	30.8181818	25.0909091	24.6363636	29.6363636	34.4545455
SD	6.72985209	5.41882241	10.4350807	7.17318238	11.4914988	8.48956579

Westland 2017 Q2

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	35	32	21	38	48	31
2	43	37	37	39	16	36
3	35	36	35	27	36	20
4	47	32	38	25	40	32
5	35	42	51	40	37	38
6	39	52	50	37	32	31
7	40	40	42	42	41	30
8	34	28	32	40	39	29
9	34	33	37	33	30	33
10	24	39	41	59	34	39
11	54	42	39	40	27	33
12	39	39	34	36	38	25
Mean	38.25	37.6666667	38.0833333	38	34.8333333	31.4166667
Std	7.52118221	6.28610782	7.92531426	8.47456086	8.08852536	5.28218848

Westland 2017 Q3

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	23	26	20	21	21	34
2	22	27	20	26	24	27
3	21	26	23	26	31	30
4	11	27	25	22	23	8
5	22	14	35	22	22	2
6	27	22	19	24	2	0
7	20	21	27	24	29	0
8	29	20	28	20	21	24
9	29	24	24	22	23	26
10	22	22	22	22	24	18
11	31	25	21	20	23	21
12	22	26	23	19	26	21
Mean	23.25	23.3333333	23.9166667	22.3333333	22.4166667	17.5833333
Std	5.32788548	3.79792607	4.46111143	2.26969495	7.11539092	12.0639081

Westland 2017 Q4

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	16	24	10	14	28	0
2	21	14	16	29	20	10
3	19	25	16	23	22	1
4	19	23	26	13	22	0
5	20	27	26	18	15	1
6	23	24	20	19	19	3
7	26	15	17	23	18	4
8	24	20	21	22	16	5
9	23	20	18	16	13	0
10	20	17	16	19	22	1
11	19	18	25	13	25	0
12	20	21	26	11	24	0
Mean	20.833333	20.666667	19.750000	18.333333	20.333333	2.083333
Std	2.724746	4.097301	5.172040	5.280037	4.376244	3.028901

Westland 2018 Q1

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	33	31	43	38	41	22
2	34	36	38	46	36	30
3	35	40	41	34	43	19
4	33	32	41	42	45	30
5	41	39	40	37	33	27
6	38	47	43	40	40	32
7	30	47	43	45	46	41
8	31	43	45	39	46	32
9	4	59	37	24	45	26
10	43	44	45	43	40	17
11	34	40	42	49	41	22
12	29	45	41	38	45	23
Mean	32.0833333	41.9166667	41.5833333	39.5833333	41.75	26.75
Std	9.7929319	7.5493327	2.46644143	6.48716682	4.11482906	6.70312003

Westland 2018 Q2

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	28	25	14	29	25	7
2	27	27	14	27	28	11
3	27	24	27	27	23	15
4	26	12	23	10	24	5
5	12	12	11	23	28	4
6	36	30	30	30	24	0
7	33	30	26	31	29	3
8	22	26	26	25	25	0
9	21	24	12	26	30	13
10	14	13	24	12	24	9
MEAN	24.6	22.3	20.7	24	26	6.7
SD	7.57481206	7.19644974	7.13442359	7.25718035	2.49443826	5.22919157

Westland 2018 Q3

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	39	33	36	34	37	32
2	42	25	43	38	43	39
3	24	41	42	46	40	33
4	41	20	35	41	35	39
5	39	26	39	38	33	31
6	46	35	43	51	42	35
7	38	31	39	39	32	43
8	36	38	43	40	40	34
9	37	18	43	41	44	34
10	6	30	30	42	46	37
11	39	20	41	39	38	33
12	36	23	40	47	37	31
MEAN	35.25	28.3333333	39.5	41.3333333	38.9166667	35.0833333
SD	10.5754862	7.51160718	4.05642028	4.63844279	4.37884031	3.70401093

Appendix C: Brandywine Major Ion Available Analytes and *Mock Synthetic Effluent* target in mg/L and mmol

In mg/L	20% Perrier	Brandywine Effluent 2017-2018					Target
		Q2	Q3-1	Q3-2	Q4	Q1	
Calcium	26	390	320	490	510		510
Chloride	4		720	920	830		920
Magnesium	1	120	79	110	150		150
Sodium	2					730	730
Potassium	ND					170	170
Sulfate	6		1300	1900	1900		1900
TDS		4400	3600	5300	4000		
Cond (µS/cm)	150	5260	4710	5800	6470	3330	
In mmol	**20% Perrier**	**Brandywine Effluent 2017-2018**					**Target**
		Q2	**Q3-1**	**Q3-2**	**Q4**	**Q1**	
Calcium	0.65	9.73	7.98	12.23	12.73		12.72
Chloride	0.11	0.99	20.31	25.95	23.41		25.94
Magnesium	0.04	4.94	3.25	4.53	6.17		6.17
Sodium	0.09					31.75	31.75
Potassium						4.35	4.35
Sulfate	0.06	0.08	13.53	19.78	19.78		19.78

Appendix D:

Lab Sample	Sample Date	Analyte	Result	Units	Goal mg/L	Goal with DMW	% Difference
Control DMW	4/11/18	Chloride	4.84	mg/L	0	4.84	
Control DMW	4/11/18	Sulfate	6.48	mg/L	0	6.48	
Control DMW	4/11/18	Calcium	35	mg/L	0	35	
Control DMW	4/11/18	Magnesium	0.87	mg/L	0	0.87	
Control DMW	4/11/18	Potassium	0.23	mg/L	0	0.23	
Control DMW	4/11/18	Selenium	ND	µg/L	0	ND	
Control DMW	4/11/18	Sodium	2.8	mg/L	0	2.8	
Control DMW	4/4/18	Alkalinity, Bicarbonate (as CaCO3)	85	mg CaCO3/L	0		
Control DMW	4/4/18	Alkalinity, Total (to pH 4.5)	85	mg CaCO3/L	0		
10% Mock	4/11/18	Chloride	117	mg/L	102	106.84	**9.51%**
10% Mock	4/11/18	Sulfate	201	mg/L	181.5	187.98	**6.93%**
10% Mock	4/11/18	Calcium	82	mg/L	51	86	**4.65%**
10% Mock	4/11/18	Magnesium	17	mg/L	15	15.87	**7.12%**
10% Mock	4/11/18	Potassium	21	mg/L	17	17.23	**21.88%**
10% Mock	4/11/18	Selenium	ND	µg/L	0	ND	**ND**
10% Mock	4/11/18	Sodium	95	mg/L	79.1	81.9	**16.00%**
10% Mock	4/4/18	Alkalinity, Bicarbonate (as CaCO3)	82	mg CaCO3/L			
10% Mock	4/4/18	Alkalinity, Total (to pH 4.5)	82	mg CaCO3/L			
18% Mock	4/11/18	Chloride	204	mg/L	183.6	188.44	**8.26%**
18% Mock	4/11/18	Sulfate	356	mg/L	326.7	333.18	**6.85%**
18% Mock	4/11/18	Calcium	91	mg/L	91.8	126.8	**28.23%**
18% Mock	4/11/18	Magnesium	25	mg/L	27	27.87	**10.30%**
18% Mock	4/11/18	Potassium	31	mg/L	30.6	30.83	**0.55%**
18% Mock	4/11/18	Selenium	ND	µg/L	0	ND	**ND**
18% Mock	4/11/18	Sodium	140	mg/L	142.38	145.18	**3.57%**

18% Mock	4/4/18	Alkalinity, Bicarbonate (as CaCO3)	81	mg CaCO3/L			
18% Mock	4/4/18	Alkalinity, Total (to pH 4.5)	81	mg CaCO3/L			
32% Mock	4/11/18	Chloride	363	mg/L	326.4	331.24	**9.59%**
32% Mock	4/11/18	Sulfate	634	mg/L	580.8	587.28	**7.96%**
32% Mock	4/11/18	Calcium	160	mg/L	163.2	198.2	**19.27%**
32% Mock	4/11/18	Magnesium	45	mg/L	48	48.87	**7.92%**
32% Mock	4/11/18	Potassium	49	mg/L	54.4	54.63	**10.31%**
32% Mock	4/11/18	Selenium	ND	µg/L	0	ND	**ND**
32% Mock	4/11/18	Sodium	240	mg/L	253.12	255.92	**6.22%**
32% Mock	4/4/18	Alkalinity, Bicarbonate (as CaCO3)	80	mg CaCO3/L			
32% Mock	4/4/18	Alkalinity, Total (to pH 4.5)	80	mg CaCO3/L			
56% Mock	4/11/18	Chloride	614	mg/L	571.2	576.04	**6.59%**
56% Mock	4/11/18	Sulfate	1070	mg/L	1016.4	1022.88	**4.61%**
56% Mock	4/11/18	Calcium	270	mg/L	285.6	320.6	**15.78%**
56% Mock	4/11/18	Magnesium	71	mg/L	84	84.87	**16.34%**
56% Mock	4/11/18	Potassium	79	mg/L	95.2	95.43	**17.22%**
56% Mock	4/11/18	Selenium	ND	µg/L	0	ND	**ND**
56% Mock	4/11/18	Sodium	390	mg/L	442.96	445.76	**12.51%**
56% Mock	4/4/18	Alkalinity, Bicarbonate (as CaCO3)	78	mg CaCO3/L			
56% Mock	4/4/18	Alkalinity, Total (to pH 4.5)	78	mg CaCO3/L			
100% Mock	4/11/18	Chloride	1150	mg/L	1020	1024.84	**12.21%**
100% Mock	4/11/18	Sulfate	2000	mg/L	1815	1821.48	**9.80%**
100% Mock	4/11/18	Calcium	410	mg/L	510	545	**24.77%**
100% Mock	4/11/18	Magnesium	160	mg/L	150	150.87	**6.05%**
100% Mock	4/11/18	Potassium	180	mg/L	170	170.23	**5.74%**
100% Mock	4/11/18	Selenium	ND	µg/L	0	ND	**ND**

100% Mock	4/11/18	Sodium	870	mg/L	791	793.8	**9.60%**
100% Mock	4/4/18	Alkalinity, Bicarbonate (as CaCO3)	75	mg CaCO3/L	610		
100% Mock	4/4/18	Alkalinity, Total (to pH 4.5)	75	mg CaCO3/L			
10% NaCl	4/11/18	Chloride	93.2	mg/L	91.9	96.84	**3.76%**
10% NaCl	4/11/18	Sulfate	9.34	mg/L	0	6.48	
100% NaCl	4/11/18	Chloride	911	mg/L	919	923.84	**1.39%**
100% NaCl	4/11/18	Sulfate	5.86	mg/L	0	6.48	
100% NaCl	4/11/18	Calcium	30	mg/L	0	35	
100% NaCl	4/11/18	Magnesium	0.85	mg/L	0	0.87	
100% NaCl	4/11/18	Potassium	0.47	mg/L	0	0.23	
100% NaCl	4/11/18	Selenium	ND	µg/L	0	ND	
100% NaCl	4/11/18	Sodium	560	mg/L	596	598.8	**6.48%**
Control DMW	4/13/18	Chloride	4.73	mg/L	0	4.73	
Control DMW	4/13/18	Sulfate	7.37	mg/L	0	7.37	
Control DMW	4/19/18	Calcium	32	mg/L	0	32	
Control DMW	4/19/18	Magnesium	0.75	mg/L	0	0.75	
Control DMW	4/19/18	Potassium	0.20	mg/L	0	0.20	
Control DMW	4/19/18	Selenium	ND	µg/L	0	ND	
Control DMW	4/19/18	Sodium	3.0	mg/L	0	3.0	
10% Na2SO4	4/13/18	Chloride	4.58	mg/L	0	4.73	
10% Na2SO4	4/13/18	Sulfate	189	mg/L	189.4	196.77	**3.95%**
10% Na2SO4	4/19/18	Calcium	36	mg/L	0	32	
10% Na2SO4	4/19/18	Magnesium	0.99	mg/L	0	0.75	
10% Na2SO4	4/19/18	Potassium	0.39	mg/L	0	0.2	
10% Na2SO4	4/19/18	Selenium	ND	µg/L	0	ND	
10% Na2SO4	4/19/18	Sodium	93	mg/L	90.6	93.6	**0.64%**
100% Na2SO4	4/13/18	Chloride	4.30	mg/L	0	4.73	

100% Na2SO4	4/13/18	Sulfate	1910	mg/L	1894	1901.37	**0.45%**
100% Na2SO4	4/19/18	Calcium	27	mg/L	0	32	
100% Na2SO4	4/19/18	Magnesium	0.64	mg/L	0	0.75	
100% Na2SO4	4/19/18	Potassium	0.28	mg/L	0	0.2	
100% Na2SO4	4/19/18	Selenium	ND	µg/L	0		
100% Na2SO4	4/19/18	Sodium	740	mg/L	906	909	**18.59%**
10% KCl	4/13/18	Chloride	19.7	mg/L	15.4	20.13	**2.14%**
10% KCl	4/13/18	Sulfate	7.13	mg/L	0	7.37	
10% KCl	4/19/18	Calcium	30	mg/L	0	32	
10% KCl	4/19/18	Magnesium	0.75	mg/L	0	0.75	
10% KCl	4/19/18	Potassium	14	mg/L	16.9	17.1	**18.13%**
10% KCl	4/19/18	Selenium	ND	µg/L	0	ND	
10% KCl	4/19/18	Sodium	3.2	mg/L	0	3	
100% KCl	4/13/18	Chloride	160	mg/L	154	158.73	**0.80%**
100% KCl	4/13/18	Sulfate	6.13	mg/L	0	7.37	
100% KCl	4/19/18	Calcium	28	mg/L	0	32	
100% KCl	4/19/18	Magnesium	0.68	mg/L	0	0.75	
100% KCl	4/19/18	Potassium	130	mg/L	169	169.2	**23.17%**
100% KCl	4/19/18	Selenium	ND	µg/L	0	ND	
100% KCl	4/19/18	Sodium	2.6	mg/L	0	3	

Appendix E: TIE Synthetic Effluent Reproduction Raw Data

4/4/18 Mock Synthetic Effluent I

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	32	40	34	45	27	0
2	41	46	40	46	34	0
3	20	48	48	48	33	0
4	53	28	49	38	3	0
5	34	37	50	42	35	0
6	44	18	37	44	25	0
7	29	48	53	27	38	0
8	47	49	47	42	23	0
9	20	47	49	38	32	0
10	25	52	42	42	13	0
11	46	50	49	37	30	0
12	41	55	49	48	28	0
mean	36.0	43.2	45.6	41.4	26.8	0.0
std dev	11.0	10.8	5.9	5.9	10.0	

4/4/18 Cl^- Synthetic Effluent

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	24	40	34	41	42	14
2	45	50	53	50	45	13
3	49	49	43	49	41	35
4	30	48	45	57	43	26
5	49	50	48	47	44	14
6	56	52	48	48	45	29
7	43	58	53	54	49	41
8	45	51	47	43	48	1
9	45	54	48	46	48	17
10	50	57	49	47	51	43
11	44	48	48	53	41	30
12	45	48	52	52	48	37
mean	43.8	50.4	47.3	48.9	45.4	25.0
stdev	8.7	4.7	5.2	4.6	3.3	13.1

4/12/18 K^+ Synthetic Effluent

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	20	34	0	39	20	0
2	37	37	38	43	23	0
3	40	29	33	29	19	0
4	36	30	16	33	17	0
5	26	34	35	38	28	0
6	43	42	62	41	38	0
7	36	41	56	29	11	0
8	36	39	38	40	29	0
9	26	45	42	36	36	0
10	36	31	33	36	44	0
11	26	49	53	37	46	0
12	25	35	22	8	25	0
Mean	32.3	37.2	35.7	34.1	28.0	0.0
St dev	7.2	6.2	17.4	9.3	11.0	0.0

4/12/18 SO_4^- Synthetic Effluent

	Treatment					
Rep	Cont	10%	18%	32%	56%	100%
1	27	36	35	29	27	13
2	51	32	36	48	36	19
3	54	61	52	35	39	25
4	36	40	59	29	45	0
5	38	41	46	31	25	13
6	42	43	39	45	32	16
7	60	53	63	42	46	25
8	39	39	46	31	28	23
9	23	29	37	44	24	23
10	38	28	36	37	28	10
11	34	41	34	32	37	23
12	46	46	51	31	34	3
mean	40.7	40.8	44.5	36.2	33.4	16.1
stdev	10.7	9.5	9.9	6.8	7.4	8.5

8/2/2018 Mock- K^+ Synthetic Effluent

Treatment						
Rep	Cont	32%	42%	56%	75%	100%
1	28	31	20	0	0	0
2	20	17	10	0	0	0
3	25	17	12	0	0	0
4	3	20	10	0	0	0
5	26	22	23	0	0	0
6	28	22	17	1	1	0
7	26	18	11	0	0	0
8	25	17	26	0	0	0
9	31	2	9	2	0	0
10	17	8	23	0	0	0
MEAN	22.9	17.4	16.1	0.3	0.1	0.0
SD	8.1	7.9	6.5	0.7	0.3	0.0

8/2/2018 K^+ Synthetic Effluent II

Treatment						
Rep	Cont	32%	42%	56%	75%	100%
1	33	5	30	0	0	0
2	24	27	14	15	0	0
3	26	32	32	0	0	0
4	27	15	17	0	0	0
5	32	31	29	0	0	0
6	25	34	33	0	0	0
7	28	27	23	23	0	0
8	24	23	23	0	0	0
9	23	22	18	0	0	0
10	24	2	24	19	0	0
MEAN	26.6	21.8	24.3	5.7	0	0
SD	3.5	11.1	6.6	9.4	0.0	0.0

8/9/2018 Mock Synthetic Effluent II

Treatment							
Rep	Cont	24%	32%	42%	56%	75%	100%
1	24	35	26	25	25	6	0
2	35	34	36	29	23	0	0
3	30	32	34	26	27	4	0
4	29	31	29	24	14	0	0
5	30	32	29	24	21	0	0
6	27	29	20	25	19	0	0
7	28	30	35	29	23	0	0
8	36	33	27	29	18	5	0
9	35	32	15	30	23	2	0
10	31	34	29	25	24	4	0
11	27	35	32	26	20	0	0
12	34	33	32	23	10	0	0
MEAN	30.5	32.5	28.7	26.3	20.6	1.8	0.0
SD	3.8	1.9	6.2	2.4	4.8	2.3	0.0

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